

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C08F 246/00, C09D 157/00	A1	(11) International Publication Number: WO 93/01221 (43) International Publication Date: 21 January 1993 (21.01.93)
(21) International Application Number: PCT/GB92/01215 (22) International Filing Date: 6 July 1992 (06.07.92) (30) Priority data: 9114619.1 5 July 1991 (05.07.91) GB 9117170.2 8 August 1991 (08.08.91) GB 9208970.5 24 April 1992 (24.04.92) GB (71) Applicant (for all designated States except US): BIOCOMPATIBLES LIMITED [GB/GB]; Brunel Science Park, Kingston Lane, Uxbridge, Middlesex UB8 3PQ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : BOWERS, Roderick, William, Jonathan [GB/GB]; Biocompatibles Limited, Brunel Science Park, Kingston Lane, Uxbridge, Middlesex UB8 3PQ (GB). JONES, Stephen, Alister [GB/GB]; STRATFORD, Peter, William [GB/GB]; CHARLES, Steven, Alexander [GB/GB]; Biocompatibles Limited, Brunel Science Park, Kingston Lane, Uxbridge, Middlesex UB8 3PQ (GB).		(74) Agents: CRESSWELL, Thomas, Anthony et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB). (81) Designated States: AU, CA, JP, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE). Published <i>With international search report.</i>
(54) Title: POLYMERIC SURFACE COATINGS (57) Abstract <p>Polymers of one or more radical polymerisable monomers which polymer has pendant groups bearing a centre of permanent positive charge and other pendant groups capable of stably binding the polymer to a surface are useful in the treatment of surfaces to render them biocompatible. The polymers may contain pendant groups which bind the polymer to a surface by physiosorption, covalent bonding or ionic interactions. Additionally reactive groups in the polymer may serve as points for attachment of ligands to the polymer when coated on a surface.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

WO 93/01221

PCT/GB92/01215

- 1 -

POLYMERIC SURFACE COATINGS

The present invention relates to new polymers, processes for producing them and processes for coating surfaces with them. The invention also provides improved processes for producing certain monomers and to certain new monomers used to obtain the polymers. The polymers are useful for coating surfaces of devices and materials which come into contact with protein-containing solutions and biological fluids, and rendering the surfaces bio- and haemocompatible. Surfaces may thus be rendered suitable for prolonged contact with living tissues and body fluids and with protein-containing solutions.

Materials used in the manufacture of separation substrates and devices, blood contacting devices contact and intraocular lenses, and other devices which are used in contact with protein-containing or biological fluids must be selected on the basis of acceptable physical and mechanical properties and compatibility with the protein-containing or biological fluid. For any given application of these materials it is usually difficult to optimise all of these considerations simultaneously and a compromise must be reached often resulting in less than optimal performance. For example, major biological problems are often encountered with materials which have otherwise optimal mechanical and physical properties. These problems often manifest themselves as undesirable deposition of biological components and in particular proteinaceous material. This protein adsorption results in blood clot formation in blood-contacting materials, the adsorption of tear components onto contact lenses resulting in deposit formation, formation of deposits on intraocular lenses

WO 93/01221

PCT/GB92/01215

- 2 -

and in separation media it results in blockage and failure of separation devices. Such effects lead to significant loss in operational performance and often complete rejection and failure of devices.

- 5 In the case of medical devices, for example prostheses and components of blood dialysis equipment, it is common practice to employ biocompatible polymers to form at least the surface of the devices to discourage protein adsorption. However, these materials are not perfect and reaction with the
- 10 living tissues still remains a problem; for example surface-induced thrombosis is still a major difficulty, particularly where large quantities of blood are contacted with a foreign surface such as in artificial lungs and kidneys. Formation of a clot in an artificial organ has a number of adverse or even
- 15 catastrophic effects including occlusion of the blood pathway in the extracorporeal system, or embolism if the clot breaks off the artificial surface and lodges in a host blood vessel. Dialysis membranes, heart valves, circulatory-assist devices, blood substitutes and artificial lungs all share this problem.
- 20 It is known that materials for use as biocompatible coatings should ideally:
- (a) be capable of reproducible manufacture as pure materials;
 - (b) be capable of being coated onto surfaces without being degraded or adversely changed;
 - 25 (c) have the requisite mechanical and permeability properties required for the specific function of the device for which they are intended;
 - (d) be sterilisable without adverse changes in, for example, permeability and mechanical or surface properties;

WO 93/01221

- 3 -

PCT/GB92/01215

- (e) not be damaged or degraded by the biological environment;
- (f) not be carcinogenic.

In applications involving direct contact with blood further restrictions exist. Materials should not:

- 5 (g) induce significant platelet adhesion;
- (h) interfere with the normal clotting mechanism; or
- (i) cause any significant damage to the cellular elements or soluble components of the blood.

There have been many attempts to prepare biocompatible, and specifically blood compatible (i.e, haemocompatible), surfaces, which do not activate the blood coagulation process and do not promote thrombus formation. Examples of such attempts include the preparation of negatively charged surfaces, such as by use of anionic polymers or suitably oriented electret polymers, preparation of surfaces coated with the natural anticoagulant heparin or synthetic heparin analogues, preparation of surfaces with inherently low surface free energy such as by use of silicone rubber, preparation of albumin-coated surfaces, and preparation of surfaces coated with compounds such as some polymethanes which are thought to adsorb albumin preferentially from blood. All of these however have had limitations.

We have now devised new film-forming polymers which can be used to coat surfaces. It has been found that these copolymers may be used to provide stable coatings on a wide variety of surfaces including, polyethylene, PVC, steel and poly(imide). The invention also provides physiadsorbable polymers which when used to coat surfaces, do not swell, to any significant extent, in aqueous environments; in some situations

WO 93/01221

PCT/GB92/01215

- 4 -

swelling in aqueous environments can reduce the stability of coatings of physiadsorbable polymers on surfaces.

The polymers which contain zwitterionic groups, mimic the zwitterionic structure of phospholipids such as phosphatidylcholine and sphingomyelin which are the major components of the outer membrane of all living cells. In this way the present invention seeks to provide a biocompatible surface on a coated substrate at which the deposition of proteins and cells at the substrate is minimised when the coated substrate comes into contact with a protein-containing solution or biological fluid.

In addition a variety of ligands may be attached to the polymers of the present invention when coated onto a substrate. Alternatively ligands may be attached to the polymers prior to coating on a substrate, e.g. when the polymer is in solution. The polymers of the present invention may therefore provide a means of attachment of such ligands. The term ligand includes, but is not limited to, specific binding agents such as immunoglobulins and associated fragments thereof such as those useful for affinity separation and diagnostic applications, photosensitive and chemisensitive moieties such as those useful for detector and sensor applications and therapeutic agents useful for clinical applications. Other ligands include peptide fragments which may be chemically linked to a polymer of the invention, such as fragments which induce cell attachment and may therefore be used to allow the polymers of the present invention to provide cell seeding.

The present invention provides a polymer of one or more radical polymerisable, preferably ethylenically unsaturated,

WO 93/01221

PCT/GB92/01215

- 5 -

monomers, which polymer has pendant groups bearing a centre of permanent positive charge and other pendant groups capable of stably binding the polymer to a surface. Such coatings bind to surfaces with good adhesion and are not removable in the environment in which the coated surfaces are used, e.g. in use as a coating on a blood-contacting surface.

Groups bearing a centre of permanent positive charge can be cationic but are most preferably zwitterionic. Such zwitterionic groups mimic the structure of the head groups of phospholipids in cells. Without wishing to be limited by this theory, it is thought that the presence of such groups at a surface renders the surface more biocompatible.

The extent to which a polymer renders a surface biocompatible may be assessed as a combination of factors such as reduction in the extent to which the surface causes blood platelet activation, protein adsorption, (for instance as judged by absorption of fibrinogen from human plasma) and reaction with C-reactive protein which is caused by the presence on the surface of isolated zwitterionic, e.g. phosphate ammonium ester groups. Preferably the polymers of the invention when coated onto a substrate, provide a reduction in platelet activation of at least 70%, more preferably at least 90%, as assessed by the assay described hereinafter compared to an untreated substrate. It is also preferred that the polymers of the invention, when coated onto a substrate, provide a reduction in fibrinogen absorption of at least 60% as assessed by the assay described hereinafter and a protein index of less than 1.5×10^{-3} compared to an untreated substrate. The protein index is defined as the ratio of the absorbance due to

WO 93/01221

PCT/GB92/01215

- 6 -

C-reactive protein measured in the assay described hereinafter to the reduction in fibrinogen adsorption.

The nature of the groups capable of binding the polymer to a surface will be selected depending upon the nature of the surface which it is intended to coat with the polymer. Where the surface is hydrophobic, groups capable of being physisorbed at the surface may be used to bind the polymer to the surface. Where the surface is hydrophilic and bears functional groups then groups which are capable of reacting with surface functional groups to form covalent bonds may be used to bind the polymer to the surface. Where the surface is charged then groups bearing ionic charge may be used to bind the polymer to the surface by ionic interactions.

Polymers of the invention may therefore bind to a surface by physisorption, covalent or ionic bonding depending upon the precise nature of the surface. In certain cases it may be possible to use two of these binding mechanisms in combination.

The groups capable of stably binding the polymer to a surface may be present in the same monomer as the groups bearing a centre of permanent positive charge, or they may be in separate monomer species which are copolymerised to provide the polymer of the invention.

It will be understood that throughout, where a group is referred to as capable of binding a polymer to a surface this is intended to mean stably binding.

Where a hydrophobic surface is to be coated, alkyl groups of 6 or more carbon atoms, or fluoroalkyl groups, optionally having one or more etheric oxygen atoms interrupting the carbon chain, and optionally containing one or more carbon-carbon

double or triple bonds, or siloxane groups, preferably containing from 1 to 50, more preferably 5 to 30, silicon atoms, may be used as the pendant groups capable of binding the polymer to a surface. Such groups are capable of forming strong secondary valence interactions with a surface, and being physisorbed at a hydrophobic surface, i.e. adsorbed without formation of a covalent interaction.

In one embodiment the present invention therefore provides a polymer obtainable by

- 10 (i) copolymerising a radical polymerisable, preferably an ethylenically unsaturated, comonomer containing a group bearing a centre of permanent positive charge, which is preferably zwitterionic, and a radical polymerisable, preferably an ethylenically unsaturated, comonomer containing a radical polymerisable moiety and an alkyl group of 6 or more carbon atoms, which alkyl group optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or a siloxane group; or

- (ii) polymerising a radical polymerisable, preferably ethylenically unsaturated, monomer containing a group bearing a centre of permanent positive charge which is preferably zwitterionic, and an alkyl group of 6 or more carbon atoms, which alkyl group optionally contains one or more etheric oxygen atoms, or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms, or a siloxane group.

Such a polymer may be a copolymer comprising residues of

WO 93/01221

PCT/GB92/01215

- 8 -

a radical polymerisable, preferably ethylenically unsaturated, comonomer containing a group bearing a centre of permanent positive charge and of a radical polymerisable, preferably ethylenically unsaturated comonomer containing, in addition to the radical polymerisable moiety, an alkyl group of 6 or more carbon atoms which group optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or a siloxane group.

Alternatively such a polymer may comprise or consist of residues of a radical polymerisable, preferably ethylenically unsaturated, monomer containing a group bearing a centre of permanent positive charge and an alkyl group of 6 or more carbon atoms which group optionally contains one or more etheric oxygen atoms or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms, or a siloxane group.

In this embodiment, preferably the polymer is a copolymer comprising residues of a comonomer containing a physisorbable group and a comonomer containing a group bearing a centre of permanent positive charge.

It is also preferred that the physisorbable group is an alkyl or fluoroalkyl group optionally containing one or more carbon-carbon double or triple bonds. Such a group may contain one or more etheric oxygen atoms, but in an especially preferred embodiment does not contain any etheric oxygen atoms.

In one embodiment, where the physisorbable group is an alkyl or fluoroalkyl group, optionally containing one or more etheric oxygen atoms, this group does not contain any carbon-

WO 93/01221

PCT/GB92/01215

- 9 -

carbon double or triple bonds.

Where a hydrophilic surface having functional groups is to be coated, groups capable of covalently binding the polymer to the surface may be incorporated into the polymer as pendant groups.

Thus according to an alternative embodiment, the invention provides a polymer obtainable by:

(i) copolymerising a radical polymerisable, preferably ethylenically unsaturated, comonomer containing a group bearing a centre of permanent positive charge, which is preferably zwitterionic, and a radical polymerisable, preferably ethylenically unsaturated, comonomer bearing a reactive group capable of covalently binding the polymer to a surface; or

(ii) polymerising a radical polymerisable, preferably ethylenically unsaturated, monomer containing a group bearing a centre of permanent positive charge, which is preferably zwitterionic, and a reactive group capable of covalently binding the polymer to a surface.

Such a polymer may be a copolymer comprising residues of a radical polymerisable, preferably ethylenically unsaturated, comonomer containing a group bearing a centre of permanent positive charge and a radical polymerisable, preferably ethylenically unsaturated, comonomer bearing a reactive group and is capable of covalently binding to a surface.

Alternatively, such a polymer may comprise or consist of residues of a radical polymerisable, preferably ethylenically unsaturated, monomer containing a group bearing a centre of permanent positive charge and a reactive group capable of covalently binding to a surface.

WO 93/01221

PCT/GB92/01215

- 10 -

In this embodiment, preferably the polymer is a copolymer comprising residues of a comonomer containing a group bearing a centre of permanent positive charge and a comonomer containing a reactive group capable of covalently binding to the surface.

5 Where a surface bearing an ionic charge is to be coated, ionic groups, capable of binding the polymer to the surface by ionic interactions, may be incorporated into the polymer of the invention as pendant groups.

According to a third embodiment, the invention therefore
10 provides a polymer obtainable by:

(i) copolymerising a radical polymerisable, preferably ethylenically unsaturated, comonomer containing a group bearing a centre of permanent positive charge which is preferably zwitterionic, and a radical polymerisable, preferably
15 ethylenically unsaturated, comonomer bearing an ionic group capable of binding to a surface by ionic interaction; or

(ii) polymerising a radical polymerisable, preferably ethylenically unsaturated, monomer containing a group bearing a centre of permanent positive charge, which is preferably
20 zwitterionic, and an ionic group capable of binding to a surface by ionic interaction.

Such a polymer may be a copolymer comprising residues of a radical polymerisable, preferably ethylenically unsaturated, comonomer containing a group bearing a centre of permanent
25 positive charge, and residues of a comonomer containing an ionic group capable of binding to a surface by ionic interaction.

Alternatively such a polymer may comprise or consist of residues of a radical polymerisable, preferably ethylenically

WO 93/01221

PCT/GB92/01215

- 11 -

unsaturated, monomer containing a group bearing a centre of permanent positive charge and an ionic group capable of binding to a surface by ionic interaction.

In this embodiment, preferably the polymer is a copolymer comprising residues of a comonomer containing a group bearing a centre of permanent positive charge and residues of a comonomer containing an ionic group capable of binding to a surface by ionic interaction.

Optionally, in any of the above embodiments, the polymers also comprise residues of one or more diluent and/or crosslinkable monomers.

The invention also provides a process for producing such a polymer which comprises polymerising such monomers and a process for coating a surface with such a polymer, for instance a process comprising the steps of (a) polymerising such monomers to form the polymer and (b) coating the surface with the polymer so formed. Optionally, the process further comprises attaching a ligand to the polymer either in solution before coating the surface, or, more preferably when coated on the surface.

In a specific embodiment the invention further provides such polymers containing residues of a crosslinkable monomer, which are uncrosslinked, when either coated on a surface or not coated on a surface and such polymers which are crosslinked when coated on a surface. The invention further provides a process of crosslinking such polymers when coated on a surface.

As yet a further feature, the present invention provides certain new monomers useful in producing the polymers of the invention.

WO 93/01221

PCT/GB92/01215

- 12 -

Monomers and comonomers which may be used in the polymers of the invention will now be described in more detail.

It is to be understood that throughout the specification (alk)acrylate, (alk)acrylic and (alk)acrylamide mean acrylate or alkacrylate, acrylic or alkacrylic and acrylamide or alkacrylamide respectively. Preferably unless otherwise stated alkacrylate, alkacrylic and alkacrylamide groups contain from 1 to 4 carbon atoms in the alkyl group thereof and are most preferably methacrylate, methacrylic or methacrylamide groups. Similarly (meth)acrylate, (meth)acrylic and (meth)acrylamide shall be understood to mean acrylate or methacrylate, acrylic or methacrylic and acrylamide or methacrylamide respectively.

Monomers Bearing A Centre of Permanent Positive Charge.

The monomer (or comonomer) bearing the centre of permanent positive charge can either be cationic or, more preferably zwitterionic. In the latter case the monomer includes within its structure not only a centre of permanent positive charge but also a centre of negative charge. Typically the centre of permanent positive charge is provided by a quaternary nitrogen atom.

Preferred comonomers which bear a centre of positive charge are of general formula (I)



wherein B is a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene chain optionally containing one or more fluorine atoms up to and including perfluorinated chains or, if X contains a carbon-carbon chain between B and the centre of permanent positive charge or if Y contains a terminal carbon atom bonded to B, a valence bond;

WO 93/01221

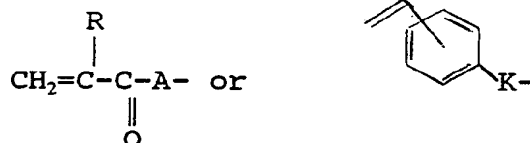
PCT/GB92/01215

- 13 -

X is a group bearing a centre of permanent positive charge, preferably a zwitterionic group and

Y is an ethylenically unsaturated polymerisable group selected from

5



10

wherein:

R is hydrogen or a C₁-C₄ alkyl group;

15 A is -O- or -NR¹- where R¹ is hydrogen or a C₁-C₄ alkyl group or R¹ is -B-X where B and X are as defined above; and

K is a group -(CH₂)_pOC(O)-, -(CH₂)_pC(O)O-,

-(CH₂)_pOC(O)O-, -(CH₂)_pNR²-, -(CH₂)_pNR²C(O)-,

-(CH₂)_pC(O)NR²-, -(CH₂)_pNR²C(O)O-, -(CH₂)_pOC(O)NR²-,

20 -(CH₂)_pNR²C(O)NR²-, (in which the groups R² are the same or

different) -(CH₂)_pO-, -(CH₂)_pSO₃ -, or, optionally in combination with B, a valence bond and p is from 1 to 12 and R² is hydrogen or a C₁-C₄ alkyl group.

The proviso on whether B may be a valence bond ensures
25 that the centre of permanent positive charge in X is not directly bonded to a heteroatom, such as an oxygen or nitrogen atom in Y.

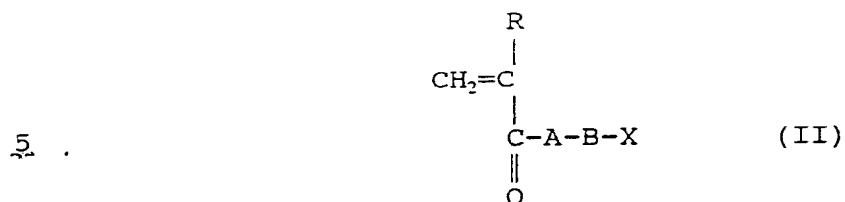
Preferred monomers containing a group bearing a centre of permanent positive charge are therefore of general formula (II)

30 or (III).

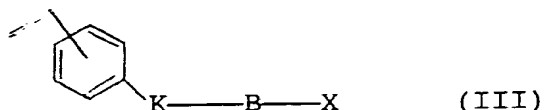
WO 93/01221

- 14 -

PCT/GB92/01215



10



15

where R, A, B, K and X are as defined with reference to formula (I) . .

Preferably in the compounds of formula (II) R is hydrogen, methyl, or ethyl, more preferably methyl, so that (II) is an acrylic acid, methacrylic acid or ethacrylic acid derivative.

In the compounds of formula (III) K may be a valence bond and B a group, K may be a group and B a valence bond, both K and B may be groups, or K and B may together be a valence bond. Preferably B is a group where K is a valence bond.

Where K is a group then preferably p is from 1 to 6, more preferably 1, 2 or 3 and most preferably p is 1. When K is a group $-(\text{CH}_2)_p\text{NR}^2-$, $-(\text{CH}_2)_p\text{NR}^2\text{C}(\text{O})-$, $-(\text{CH}_2)_p\text{C}(\text{O})\text{NR}^2$, $-(\text{CH}_2)_p\text{NR}^2\text{C}(\text{O})\text{O}-$, $-(\text{CH}_2)_p\text{OC}(\text{O})\text{NR}^2-$ or $-(\text{CH}_2)_p\text{NR}^2\text{C}(\text{O})\text{NR}^2-$ then R^2 is preferably hydrogen, methyl or ethyl, more preferably hydrogen.

In the compounds of formula (III) preferably the vinyl group is para to the group -K-B-X.

Preferably B is:

an alkylene group of formula $-(\text{CR}^3_2)_n-$, wherein the groups $-(\text{CR}^3_2)-$ are the same or different, and in each group $-(\text{CR}^3_2)-$ the groups R^3 are the same or different and each group R^3 is

hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl, preferably hydrogen, and a is from 1 to 12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6 carbon atoms in each alkyl moiety, more preferably

5 $-CH_2O(CH_2)_4-$; or

an oligo-oxaalkylene group of formula

$-[(CR^4)_2O]_c(CR^4)_b-$ where the groups $-(CR^4)_2-$ are the same or different and in each group $-(CR^4)_2-$ the groups R^4 are the same or different and each group R^4 is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl, preferably hydrogen, and b is from 1 to 6, preferably 2 or 3 and c is from 2 to 11, preferably 2 to 5; or

if X contains a carbon-carbon chain between B and the centre of permanent positive charge or if Y contains a terminal carbon atom, a valence bond.

Preferred groups B include alkylene, oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon atoms optionally containing one or more fluorine atoms. Where the polymer is not intended for coating a hydrophobic surface, and therefore is not intended to be bound by physiosorption to a surface, then preferably B is an alkylene, oxaalkylene or oligo-oxaalkylene group which does not contain any fluorine atoms.

In compounds of formula (III) it is preferred that K and B contain up to 12 carbon atoms in total.

Preferred groups X containing a centre of permanent positive charge, are the groups of formula (IVA), (IVB), (IVC), (IVD), (IVE) and (IVF) as defined below: monomers containing such groups may be used in combination with further monomers containing groups capable of binding to a surface, to provide a

WO 93/01221

PCT/GB92/01215

- 16 -

copolymer of the invention. Of these groups of formula (IVB)-
(IVF) and especially (IVC) are particularly preferred.

In addition, groups of formula (VA), (VB) and (VC) are
preferred as monomers containing both a centre of permanent
5 positive charge and an alkyl, fluoroalkyl or siloxane group
capable of binding to a surface by physisorption.

The groups of formula (IVA) are:



where the groups R^5 are the same or different and each is
10 hydrogen or C_{1-4} alkyl and Z^{\ominus} is a counter ion.

Preferably the groups R^5 are all the same. It is also
preferable that at least one of the groups R^5 is methyl, and
more preferable that all the groups R^5 are methyl.

The counterion Z^{\ominus} present in the compounds of formula (II)
15 or (III) containing a group of formula (IVA) is such that the
compounds are neutral salts. The counterion may be exchanged
with ions in physiological fluids and thus the specific nature
of the counterion is not critical in the present invention.
However, physiologically acceptable counterions are preferred.
20 Suitable physiologically acceptable counterions include halide
anions, such as chloride or bromide ions, other inorganic
anions such as sulphate, phosphate and phosphite and organic
anions such as aliphatic mono-, di- or tri-carboxylate anions
containing from 2 to 25 carbons atoms and optionally bearing
25 one or more hydroxyl groups e.g. acetate, citrate and lactate.

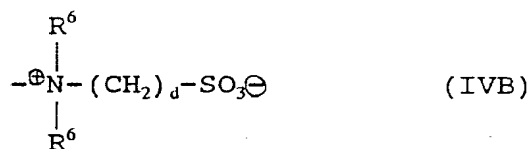
When X is a group of formula (IVA), preferably B is a
group of formula $-(\text{CR}^3)_2-$ or $-(\text{CR}^3)_2-$, eg. $-(\text{CH}_2)-$ or
 $-(\text{CH}_2\text{CH}_2)-$.

The groups of formula (IVB) are:

WO 93/01221

- 17 -

PCT/GB92/01215



5.

where the groups R^6 are the same or different and each is hydrogen or C_{1-4} alkyl and d is from 2 to 4.

Preferably the groups R^6 are the same. It is also
10 preferable that at least one of the groups R^6 is methyl, and more preferable that the groups R^6 are both methyl.

Preferably d is 2 or 3, more preferably 3.

When X is a group of formula (IVB) preferably B is a group of formula $-(\text{CR}^3)_2-$ or $-(\text{CR}^3)_2-$,

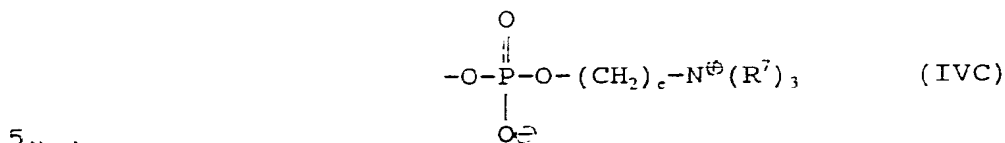
15 eg. $-(\text{CH}_2)-$ or $-(\text{CH}_2\text{CH}_2)-$.

The groups of formula (IVC) are:

WO 93/01221

- 18 -

PCT/GB92/01215



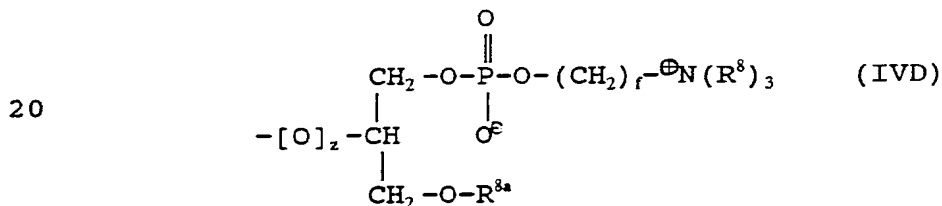
where the groups R^7 are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4.

Preferably the groups R^7 are the same. It is also
10 preferable that at least one of the groups R^7 is methyl, and more preferable that the groups R^7 are all methyl.

Preferably e is 2 or 3, more preferably 2.

When X is a group of formula (IVC) preferably B is a
group of formula $-(\text{CR}^3_2)-$ or $-(\text{CR}^3_2)_2-$,
15 eg. $-(\text{CH}_2)-$ or $-(\text{CH}_2\text{CH}_2)-$.

The groups of formula (IVD) are:



25 wherein the groups R^8 are the same or different and each is hydrogen or C_{1-4} alkyl, R^{8a} is hydrogen or, more preferably, a group $-\text{C}(\text{O})\text{B}^1\text{R}^{8b}$ where R^{8b} is hydrogen or methyl, preferably methyl, B^1 is a valence bond or straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and f is from 1 to 4;
30 and

if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0, if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1.

Preferably the groups R^8 are the same. It is also
35 preferable that at least one of the groups R^8 is methyl, and more preferable that the groups R^8 are all methyl.

Preferably f is 1 or 2, more preferably 2.

Preferably B¹ is:

a valence bond;

an alkylene group of formula $-(CR^{3a})_{aa}-$, wherein the
5 groups $-(CR^{3a})-$ are the same or different, and in each group $-(CR^{3a})-$ the groups R^{3a} are the same or different and each group R^{3a} is hydrogen or C₁₋₄ alkyl, preferably hydrogen, and aa is from 1 to 12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6
10 carbon atoms in each alkyl moiety, more preferably $-CH_2O(CH_2)_4-$; or

an oligo-oxaalkylene group of formula
 $-[(CR^{4a})_{ba}O]_{ca}-$ where the groups $-(CR^{4a})-$ are the same or
different and in each group $-(CR^{4a})-$ the groups R^{4a} are the same
15 or different and each group R^{4a} is hydrogen or C₁₋₄ alkyl,
preferably hydrogen, and ba is from 1 to 6, preferably 2 or 3,
and ca is from 1 to 12, preferably 1 to 6.

Preferred groups B¹ include a valence bond and alkylene,
oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon
20 atoms.

Preferably B and B¹ are the same.

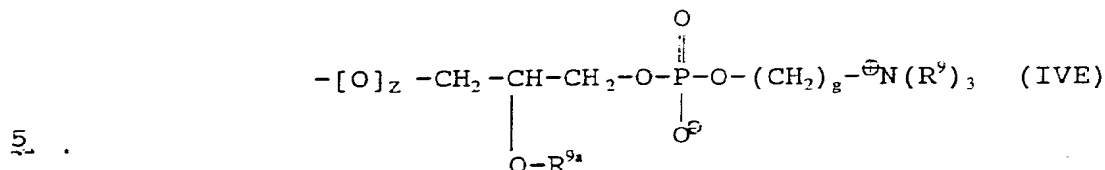
When X is a group of formula (IVD) preferably B is a
group of formula $-[(CR^4_2CR^4_2)_cO_b]CR^4_2CR^4_2-$, eg.
 $-(CH_2CH_2O)_c(CH_2CH_2)-$.

25 The groups of formula (IVE) are:

WO 93/01221

PCT/GB92/01215

- 20 -



10 wherein the groups R^9 are the same or different and each is hydrogen or C_1 - C_4 alkyl, R^{9a} is a hydrogen or, more preferably, a group $-\text{C}(\text{O})\text{B}^2\text{R}^{9b}$, R^{9b} is hydrogen or methyl, preferably methyl, B^2 is a valence bond or a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and g is from 1 to 4;
15 and

if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0 if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1.

Preferably the groups R^9 are the same. It is also
20 preferable that at least one of the groups R^9 is methyl, and more preferable that the groups R^9 are all methyl.

Preferably g is 1 or 2, more preferably 2.

Preferably B^2 is:

a valence bond;

25 an alkylene group of formula $-(\text{CR}^{3b}_2)_{ab}-$, wherein the groups $-(\text{CR}^{3b}_2)-$ are the same or different, and in each group $-(\text{CR}^{3b}_2)-$ the groups R^{3b} are the same or different and each group R^{3b} is hydrogen or C_{1-4} alkyl, preferably hydrogen, and ab is from 1 to 12, preferably 1 to 6;

30 an oxaalkylene group such as alkoxyalkyl having 1 to 6, carbon atoms in each alkyl moiety, more preferably $-\text{CH}_2\text{O}(\text{CH}_2)_4-$; or

an oligo-oxaalkylene group of formula

WO 93/01221

PCT/GB92/01215

- 21 -

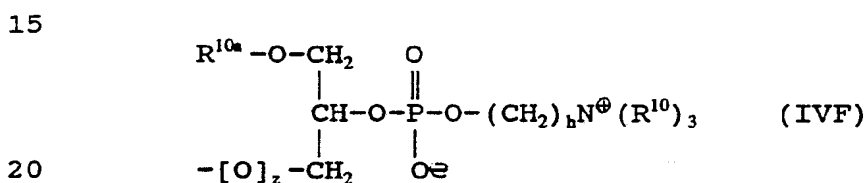
$-\left[(\text{CR}^{4b})_{bb}\text{O}\right]_{cb}-$ where the groups $-(\text{CR}^{4b})-$ are the same or different and in each group $-(\text{CR}^{4b})-$ the groups R^{4b} are the same or different and each group R^{4b} is hydrogen or C_{1-4} alkyl, preferably hydrogen, and bb is from 1 to 6, preferably 2 or 3, and cb is from 1 to 12, preferably 1 to 6.

Preferred groups B^2 include a valence bond and alkylene, oxalkylene and oligo-oxalkylene groups of up to 12 carbon atoms.

Preferably B and B^2 are the same.

When X is a group of formula (IVE) preferably B is a group of formula $-\left[(\text{CR}^4_2\text{CR}^4_2)_b\text{O}\right]_c\text{CR}^4_2\text{CR}^4_2-$, eg. $-(\text{CH}_2\text{CH}_2\text{O})_c\text{CH}_2\text{CH}_2-$.

The groups of formula (IVF) are:



wherein the groups R^{10} are the same or different and each is hydrogen or C_{1-4} alkyl, R^{10a} is hydrogen or, more preferably, a group $-\text{C}(\text{O})\text{B}^3\text{R}^{10b}$ where R^{10b} is hydrogen or methyl, preferably methyl, B^3 is a valence bond or a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and h is from 1 to 4; and

if B is other than a valence bond z is 1 and if B is a valence bond z is 0 if X is directly bonded to the oxygen or nitrogen and otherwise z is 1.

Preferably the groups R^{10} are the same. It is also preferable that at least one of the groups R^{10} is methyl, and more preferable that the groups R^{10} are all methyl.

WO 93/01221

PCT/GB92/01215

- 22 -

Preferably h is 1 or 2, more preferably 2.

Preferably B³ is:

a valence bond;

an alkylene group of formula $-(CR^{3c})_{ac}-$, wherein the
5 groups $-(CR^{3c})-$ are the same or different, and in each group $-(CR^{3c})-$ the groups R^{3c} are the same or different and each group R^{3c} is hydrogen or C₁₋₄ alkyl, preferably hydrogen, and ac is from 1 to 12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6
10 carbon atoms in each alkyl moiety, more preferably $-CH_2O(CH_2)_4-$; or

an oligo-oxaalkylene group of formula $-[(CR^{4c})_{bc}O]_{cc}-$ where the groups $-(CR^{4c})-$ are the same or different and in each group $-(CR^{4c})-$ the groups R^{4c} are the same
15 or different and each group R^{4c} is hydrogen or C₁₋₄ alkyl, preferably hydrogen, and bc is from 1 to 6, preferably 2 or 3, and cc is from 1 to 12, preferably 1 to 6.

Preferred groups B³ include a valence bond and alkylene, oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon
20 atoms.

Preferably B and B³ are the same.

When X is a group of formula (IVF) preferably B is a group of formula $-[(CR^4_2CR^4_2)_bO]_cCR^4_2CR^4_2-$, eg.
 $-(CH_2CH_2O)_cCH_2CH_2-$.

25 Further groups bearing a centre of permanent positive charge are of formula (VA), (VB) and (VC). These groups also contain an alkyl or fluoroalkyl group capable of binding to a surface by physisorption. Monomers containing such a group are therefore particularly suitable for use in the polymers of the

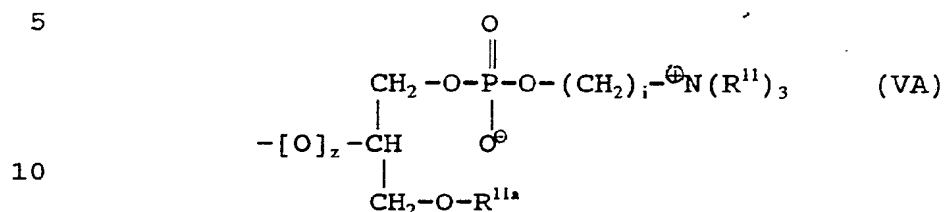
WO 93/01221

- 23 -

PCT/GB92/01215

invention, optionally without separate comonomers containing a group capable of binding to a hydrophobic surface by physisorption.

The groups of formula (VA) are:



wherein the groups R^{11} are the same or different and each is hydrogen or C_{1-4} alkyl, R^{11a} is either

(a) a group $-\text{C}(\text{O})]_{vw} (\text{CR}^{11b})_{ww} (\text{SiR}^{11c})_w (\text{OSiR}^{11c})_w R^{11c}$ in which each group R^{11b} is the same or different and is hydrogen or alkyl of 1 to 4 carbon atoms, each group R^{11c} is the same or different and is alkyl of 1 to 4 carbon atoms or aralkyl, for example benzyl or phenethyl, vw is 0 or 1, ww is from 0 to 6 with the proviso that vw and ww are not both 0, and vv is from 0 to 49;

(b) a group of formula $-\text{C}(\text{O})\text{B}^4 - R^{11d}$, in which R^{11d} is hydrogen or methyl, B^4 is a valence bond or straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group optionally containing one or more fluorine atoms, and containing from 6 to 24, preferably 6 to 18 carbon atoms; i is from 1 to 4; and

if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0 if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1.

Preferably the groups R^{11} are the same. It is also

WO 93/01221

- 24 -

PCT/GB92/01215

preferable that at least one of the groups R^{11} is methyl, and more preferable that the groups R^{11} are all methyl.

Preferably i is 1 or 2, more preferably 2.

Where R^{11a} is a siloxane group as defined in (a) above,
5 each group $(CR^{11b})_2$ may be the same or different, preferably the same, and preferably each group R^{11b} is hydrogen. Preferably w is from 2 to 4, and is most preferably 3 when v is 0 or 2 when v is 1. Each group $(SiR^{11c})_2$ may be the same or different, preferably the same, and preferably each group R^{11c} is methyl.
10 Preferably v is from 4 to 29.

Preferably the group R^{11a} is a group $-C(O)B^4R^{11d}$ as defined above. In such a case, preferably B^4 is:

a valence bond;

an alkylene group of formula $-(CR^{3d})_{ad}-$, wherein the
15 groups $-(CR^{3d})_2-$ are the same or different, and in each group $-(CR^{3d})_2-$ the groups R^{3d} are the same or different and each group R^{3d} is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl, preferably hydrogen or fluorine, and ad is from 1 to 24, preferably 6 to 18;

20 an oxaalkylene group such as alkoxyalkyl having 1 to 6 carbon atoms and optionally one or more fluorine atoms in each alkyl moiety, or

an oligo-oxalkylene group of formula
 $-[(CR^{4d})_{bd}O]_{cd}-$ where the groups $-(CR^{4d})_2-$ are the same or
25 different and in each group $-(CR^{4d})_2-$ the groups R^{4d} are the same or different and each group R^{4d} is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl, preferably hydrogen or fluorine, and bd is from 2 to 6, preferably 3 or 4, and cd is from 1 to 12, preferably 1 to 6.

When B^4 is a group $-[(CR^{4d})_{bd}O]_{cd}-$ wherein all the groups R^{4d} are hydrogen and in all the groups $-[(CR^{4d})_{bd}O]-$ bd is 2, the residues of the monomer of formula (VA) are not able to form strong secondary valence interactions with hydrophobic surfaces. Whilst residues of such monomers may be included in the polymers of the invention, it is usually also necessary to include residues of monomers which are capable of forming strong secondary valence interactions if such interactions are to bind a polymer to a surface.

Monomers which have groups containing oligo(higher alkylene) oxide moieties can be used to provide strong secondary valence interactions, so can monomers which contain oligo alkylene oxide moieties in which at least 50, preferably 70, more preferably 90 mol% of individual alkylene oxide units contain 3 or more carbon atoms. Thus, for instance a mixed oligo(ethylene oxide/propylene oxide) side chain could be used provided that there are more propylene oxide units than ethylene oxide units.

When B^4 is a group $-[(CR^4)_{bd}O]_{cd}-$ then preferably bd is 2 in only 50, preferably 70, more preferably 90 mole% or less of the residues $-[(CR^{4d})_{bd}O]-$.

When the group $-B^4-R^{11a}$ is a group capable of forming strong secondary valence interactions with a surface, then monomers containing a group (VA) may be particularly suitable for use as monomers containing a group bearing a centre of permanent positive charge and an alkyl or fluoroalkyl group optionally containing one or more etheric oxygen atoms. Preferably, in such a case $-B^4-R^{11a}$ is an alkyl group optionally

WO 93/01221

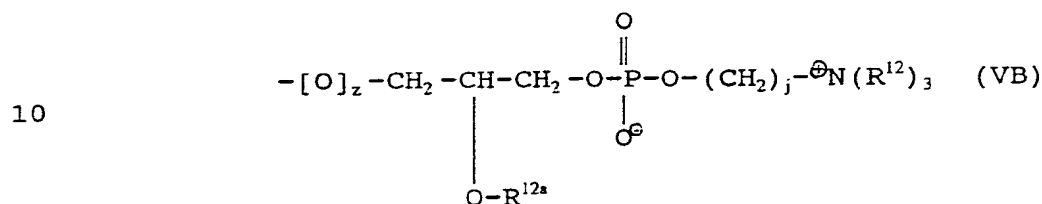
PCT/GB92/01215

- 26 -

containing one or more etheric oxygen atoms and preferably 6 or more carbon atoms or a fluoroalkyl group optionally containing one or more etheric oxygen atoms and preferably 6 or more carbon atoms.

5 In one embodiment B and B⁴ may be the same.

The groups of formula (VB) are:



wherein the groups R¹² are the same or different and each is hydrogen or C₁-C₄ alkyl, R^{12a} is either

(a) a group $-\text{[C(O)]}_m (\text{CR}^{12b})_{uu} (\text{SiR}^{12c})_t (\text{OSiR}^{12c})_n \text{R}^{12c}$ in
20 which each group R^{12b} is the same or different and is hydrogen or alkyl of 1 to 4 carbon atoms, each group R^{12c} is the same or different and is alkyl of 1 to 4 carbon atoms or aralkyl, for example benzyl or phenethyl, t is 0 or 1, uu is from 0 to 6, with the proviso that tu and uu are not both 0, and tt is from
25 0 to 49; or

(b) a group of formula $-\text{C(O)B}^5 - \text{R}^{12d}$, in which
R^{12d} is hydrogen or methyl, B⁵ is a valence bond or a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group
optionally containing one or more fluorine atoms and from 6 to
30 24 carbon atoms, more preferably 6 to 18 carbons atoms,

j is from 1 to 4; and

if B is other than a valence bond, Z is 1 and if B is a valence bond Z is 0 if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1.

WO 93/01221

PCT/GB92/01215

- 27 -

Preferably the groups R^{12} are the same. It is also preferable that at least one of the groups R^{12} is methyl, and more preferable that the groups R^{12} are all methyl.

Preferably j is 1 or 2, more preferably 2.

5 Where R^{12a} is a siloxane group as defined in (a) above, each group (CR^{12b}_2) may be the same or different, preferably the same, and preferably each group R^{12b} is hydrogen. Preferably uu is from 2 to 4, and is most preferably 3 when tu is 0 or 2 when tu is 1. Each group (SiR^{12c}_2) may be the same or different, 10 preferably the same, and preferably each group R^{12c} is methyl. Preferably tt is from 4 to 29.

Preferably the group R^{12a} is a group $-C(O)B^4R^{12d}$ as defined above. In such a case, preferably B^5 is:

a valence bond;

15 an alkylene group of formula $-(CR^{3e}_2)_{ae}-$, wherein the groups $-(CR^{3e}_2)-$ are the same or different, and in each group $-(CR^{3e}_2)-$ the groups R^{3e} are the same or different and each group R^{3e} is hydrogen, fluorine or C_{1-4} alkyl, or fluoroalkyl, preferably hydrogen or fluorine, and ae is from 1 to 24, 20 preferably 6 to 18;

an oxaalkylene group such as alkoxyalkyl having 1 to 6 carbon atoms and optionally one or more fluorine atoms in each alkyl moiety; or

an oligo-oxaalkylene group of formula

25 $-[(CR^{4e}_2)_{be}O]_{ce}-$ where the groups $-(CR^{4e}_2)-$ are the same or different and in each group $-(CR^{4e}_2)-$ the groups R^{4e} are the same or different and each group R^{4e} is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl, preferably hydrogen or fluorine, and be is from 2 to 6, preferably 3 or 4, and ce is from 1 to 12,

WO 93/01221

PCT/GB92/01215

- 28 -

preferably 1 to 6.

When B^5 is a group $-[(CR^{4c})_{bc}O]_{cc}-$ wherein all the groups R^{4c} are hydrogen and in all the groups $[(CR^{4c})_{bc}O]$ bc is 2, the residues of the monomer of formula (VB) are not able to form strong secondary valence interactions with hydrophobic surfaces. Whilst residues of such monomers may be included in the polymers of the invention, it is also necessary to include residues of monomers which are capable of forming such strong secondary valence interactions if such interactions are to bind a polymer to a surface. Monomers which have groups containing oligo(higher alkylene) oxide moieties can be used to provide the necessary strong secondary valence interactions, so can monomers which contain oligo alkylene oxide moieties in which at least 50, preferably 70, more preferably 90 mol% of individual alkylene oxide units contain 3 or more carbon atoms. Thus, for instance a mixed oligo(ethylene oxide/propylene oxide) side chain could be used provided that there are more propylene oxide units than ethylene oxide units.

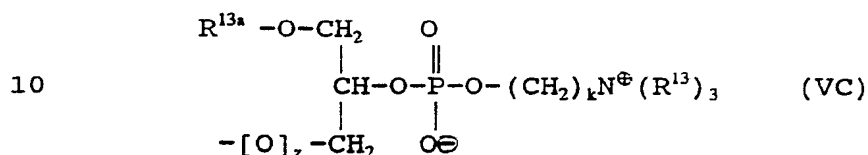
When B^5 is a group $-[(CR^{4c})_{bc}O]_{cc}-$ then preferably bc is 2 in only 50, preferably 70, more preferably 90 mole% or less of the residues $-[(CR^{4b})_{bc}O]-$.

When the group $-B^5-R^{12a}$ is a group capable of forming strong secondary valence interactions with a surface, then monomers containing a group (VB) may be particularly suitable for use as monomers containing a group bearing a centre of permanent positive charge and an alkyl or fluoroalkyl group optionally containing one or more etheric oxygen atoms. Preferably, in such a case $-B^5-R^{12a}$ is an alkyl group optionally containing one or more etheric oxygen atoms and preferably 6 or

more carbon atoms or a fluoroalkyl group optionally containing one or more etheric oxygen atoms and preferably 6 or more carbon atoms.

In one embodiment B and B⁵ may be the same.

5 The groups of formula (VC) are:



wherein the groups R¹³ are the same or different and each is
15 hydrogen or C₁₋₄ alkyl, R^{13a} is either

(a) a group $\text{---[C(O)]}_n(\text{CR}^{13b})_{rs}(\text{SiR}^{13c})_r(\text{OSiR}^{13c})_r\text{R}^{13c}$ in which each group R^{13b} is the same or different and is hydrogen or alkyl of 1 to 4 carbon atoms, each group R^{13c} is the same or different and is alkyl of 1 to 4 carbon atoms or aralkyl, for
20 example benzyl or phenethyl, rs is 0 or 1, ss is from 0 to 6, with the proviso that rs and ss are not both 0, and rr is from 0 to 49; or

(b) a group of formula $\text{---C(O)B}^6\text{---R}^{13d}$, in which R^{13a} is hydrogen or methyl, B⁶ is a valence bond or a straight or
25 branched alkylene, oxaalkylene or oligo-oxaalkylene group optionally containing one or more fluorine atoms and from 6 to 24, more preferably 6 to 18 carbon atoms and k is from 1 to 4; and

if B is other than a valence bond, Z is 1 and if B is a
30 valence bond Z is 0 if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1.

Preferably the groups R¹³ are the same. It is also preferable that at least one of the groups R¹³ is methyl, and

WO 93/01221

- 30 -

PCT/GB92/01215

more preferable that the groups R^{13} are all methyl.

Preferably k is 1 or 2, more preferably 2.

Where R^{13a} is a siloxane group as defined in (a) above,
each group (CR^{13c}_2) may be the same or different, preferably the
5 same and preferably each group R^{13b} is hydrogen. Preferably ss
is from 2 to 4, and is most preferably 3 when rs is 0 or 2 when
 rs is 1. Each group (SiR^{13c}_2) may be the same, or different,
preferably the same, and preferably each group R^{13c} is methyl.
Preferably rr is from 4 to 29.

10 Preferably the group R^{13a} is a group $-C(O)B^6R^{13d}$ as defined
above. In such a case, preferably B^6 is:

a valence bond;

an alkylene group of formula $-(CR^{3f}_2)_a-$, wherein the
groups $-(CR^{3f}_2)-$ are the same or different, and in each group -
15 $(CR^{3f}_2)-$ the groups R^{3f} are the same or different and each group
 R^{3f} is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl,
preferably hydrogen or fluorine, and is from 1 to 24,
preferably 6 to 18;

an oxaalkylene group such as alkoxyalkyl having 1 to 6
20 carbon atoms and optionally one or more fluorine atoms in each
alkyl moiety; or

an oligo-oxaalkylene group of formula
 $-[(CR^{4f}_2)_{bf}O]_{cf}-$ where the groups $-(CR^{4f}_2)-$ are the same or
different and in each group $-(CR^{4f}_2)-$ the groups R^{4f} are the same
25 or different and each group R^{4f} is hydrogen, fluorine or C_{1-4}
alkyl or fluoroalkyl, preferably hydrogen or fluorine, and bf
is from 2 to 6, preferably 3 or 4, and cf is from 1 to 12,
preferably 1 to 6.

When B^6 is a group $-[(CR^{4f}_2)_{bf}O]_{cf}-$ wherein all the groups R^{4f}

WO 93/01221

PCT/GB92/01215

- 31 -

are hydrogen and in all the groups $[(CR^{4c}_2)_{bf}O]$ bf is 2, the residues of the monomer of formula (VC) are not able to form strong secondary valence interactions with hydrophobic surfaces. Whilst residues of such monomers may be included in the polymers of the invention, it is also necessary to include residues of monomers which are capable of forming such strong secondary valence interactions if such interactions are to bind a polymer to a surface. Monomers which have groups containing oligo(higher alkylene) oxide moieties can be used to provide the necessary strong secondary valence interactions, so can monomers which contain oligo alkylene oxide moieties in which at least 50, preferably 70, more preferably 90 mol% of individual alkylene oxide units contain 3 or more carbon atoms. Thus, for instance a mixed oligo(ethylene oxide/propylene oxide) side chain could be used provided that these are more propylene oxide units than ethylene oxide units.

When B^6 is a group $-[(CR^{4f}_2)_{bf}O]_{cf}-$ then preferably bf is 2 in only 50, preferably 70, more preferably 90 mol% or less of the residues $-[(CR^{4f}_2)_{bf}O]-$.

When the group $-B^6-R^{13a}$ is a group capable of forming strong secondary valence interactions with a surface, then monomers containing a group (VC) may be particularly suitable for use as monomers containing a group bearing a centre of permanent positive charge and an alkyl or fluoroalkyl group optionally containing one or more etheric oxygen atoms. Preferably, in such a case $-B^6-R^{13a}$ is an alkyl group optionally containing one or more etheric oxygen atoms and preferably 6 or more carbon atoms or a fluoroalkyl group optionally containing one or more etheric oxygen atoms and preferably 6 or more

WO 93/01221

PCT/GB92/01215

- 32 -

carbon atoms.

In one embodiment B and B⁶ may be the same.

Particular examples of preferred monomers bearing a group containing a centre of permanent positive charge are

5 2(methacryloyloxy)ethyl-2'(trimethylammonium)ethyl phosphate inner salt and 1[4(4'-vinylbenzyloxy)butane]-2''(trimethylammonium)ethyl phosphate inner salt.

Monomers bearing a group containing a centre of permanent positive charge, such as those of formula (II) and (III) may be prepared by conventional techniques using known reactions, for

10 example using a suitable substituted alkyl (alk)acrylate or suitable substituted styrene as precursor. Examples of suitable substituted alkyl (alk)acrylates include dimethylaminoethyl(meth)acrylate and 2-

15 hydroxyethyl(meth)acrylate.

Monomers of formula (II) or (III) containing a group of formula (IVA) may be prepared by known methods. Monomers containing a group of formula (IVB) or (IVC) may be prepared as described in Reference Example 1 to 3 or by analogous known

20 methods.

Monomers of formula (II) or (III) containing a group of formula (IVD) in which R^{8a} is -C(O)B¹R^{8b} may be prepared by selective acylation of glycerophosphorylcholine or analogues thereof at the primary hydroxyl group with an activated acid

25 derivative such as an acid anhydride O(C(O)B¹R^{8b})₂ or an acid halide R^{8b}B¹COHal where B¹ and R^{8b} are as defined above and Hal is halogen, followed by acylation of the secondary hydroxyl group with an appropriate acylating agent, for example methacryloyl chloride. Purification, for example by column chromatography

WO 93/01221

PCT/GB92/01215

- 33 -

on a suitable support, may be performed after each acylation or after the second acylation only. Suitable activated acid derivatives include acid anhydrides, acid halides, reactive esters and imidazolides. The acylations may be performed in a suitable anhydrous, aprotic solvent, for example N,N-dimethylformamide, optionally in the presence of a suitable non-nucleophilic base, for example triethylamine.

Alternatively, the primary alcohol group in glycerophosphoryl choline or an analogue thereof may be blocked by reaction with a suitable protecting group reagent, for example t-butyldimethylsilyl chloride, under standard conditions and the secondary hydroxy group then treated with an acylating agent such as methacryloyl chloride. The t-butyldimethylsilyl protecting group may be removed by treatment with a dilute organic or mineral acid, for example p-toluene sulphonic acid, hydrochloric acid or with tetra-butylammonium fluoride. The deblocked primary hydroxyl group may then be treated with an activated acid derivative such as an acid anhydride $O(C(O)B^1R^{8b})_2$ or acid halide $R^{8b}B^1COHal$ where B^1 and R^{8b} are as defined above, and Hal is halogen.

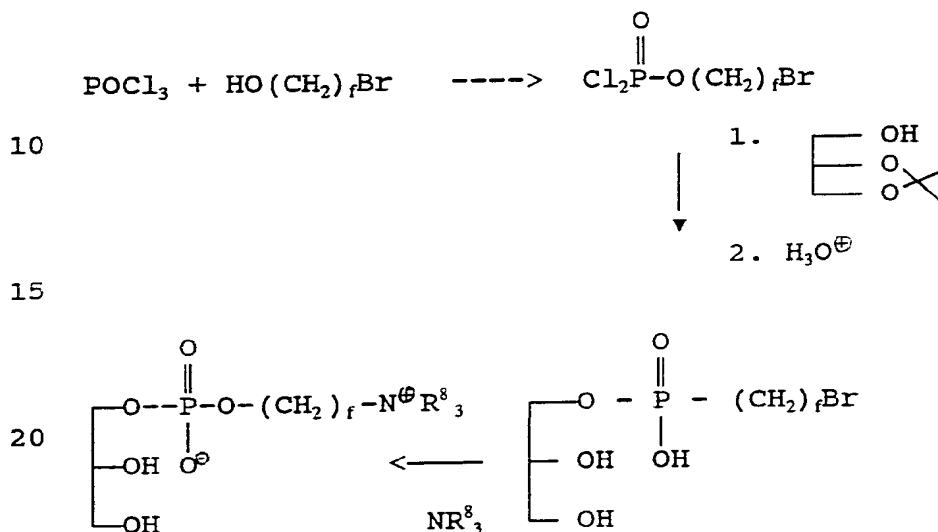
Analogues of glycerophosphorylcholine (compounds of formula (II) or (III) containing a group (IVD) where R^{8a} is hydrogen) may be prepared by reaction of phosphorus oxychloride with a bromoalcohol in an inert aprotic solvent, such as dichloromethane, to give a bromoalkylphosphorodichloridate. The dichloro derivative thus produced may then be treated with an appropriately protected glycerol derivative, for example 2,2-dimethyl 1,3-dioxolane-4-methanol, in the presence of a base, for example triethylamine,

WO 93/01221

PCT/GB92/01215

- 34 -

followed by acid hydrolysis to give a bromoalkylphosphoro-
glycerol derivative. This may then be treated with an amine
~NR⁸, where R⁸ is as defined above, for example trimethylamine,
to generate the glycerophosphorylcholine analogue. This
5 preparation is depicted in the following scheme.



25 where R⁸ and f are as defined in relation to groups of
formula (IVD).

Monomers of formula (II) or (III) containing a group of
formula (IVE) in which R^{9a} is -C(O)B²R^{9b} may be prepared by the
30 selective acylation of glycerophosphorylcholine or an analogue
thereof at the primary hydroxyl group with for example,
methacryloyl chloride followed by reaction at the secondary
hydroxyl group using an activated acid derivative, such as an
acid halide O(C(O)B²R^{9b})₂ or an acid halide R^{9b}B²COHal, where B²
35 and R^{9b} are as defined above and Hal is halogen. The
intermediates and final products may be purified, as necessary
using column chromatography. Optionally, protecting group
strategy, similar to that outlined above in relation to
production of monomers containing a group of formula (IVD) may

be employed.

Monomers of formula (II) or (III) containing a group of formula (IVF) may be prepared in an analogous manner to monomers containing groups of formula (IVD) or (IVE).

5 Monomers of formula (II) or (III) containing a group of formula (VA), (VB) or (VC) may be prepared by direct analogy with methods described for monomers containing groups of formula (IVD), (IVE) and (IVF) respectively.

Comonomers capable of stably binding a polymer to a
10 surface

In the polymer of the invention, where the group bearing a centre of permanent positive charge and group capable of stably binding the polymer to a surface are not present in the residue of the same monomer, the polymer comprises residues of
15 comonomer containing a group capable of stably binding a polymer to a surface as well as the residues of the comonomer containing a group bearing a centre of permanent positive charge. Optionally, where the monomer containing a group bearing a centre of permanent positive charge also contains a
20 group capable of stably binding the polymer to a surface, further groups capable of stably binding to a surface may be provided by additional comonomer residues containing a group capable of binding the polymer to a surface.

As has already been mentioned, the nature of the group
25 capable of binding to a surface, and therefore the nature of the comonomers containing such groups, will depend upon the nature of the surface which is to be coated with the polymer. The various types of such comonomers will now be described.

WO 93/01221

PCT/GB92/01215

- 36 -

It will be appreciated that in some circumstances it may be desirable to use a combination of different comonomers containing groups capable of binding to a surface. Preferably a comonomer of type a), b) and/or c) as defined below or a combination of such comonomers is used, more preferably only one of comonomer types a), b) and c) is used.

a) Comonomers containing an alkyl, fluoroalkyl or siloxane group

The comonomers containing an alkyl, fluoroalkyl or siloxane group, which are suitable for providing binding to a hydrophobic surface, are comonomers containing an alkyl group of 6 or more carbon atoms which group optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds or a fluoroalkyl group, preferably of 6 or more carbon atoms, which group optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or containing a siloxane group, containing up to 50 silicon atoms, preferably in a linear chain.

Preferably the alkyl or fluoroalkyl groups contains up to 24 carbon atoms, for instance up to 18 carbon atoms, or containing a siloxane group, containing up to 50 silicon, preferably in a linear chain. Preferred comonomers containing an alkyl, fluoroalkyl or siloxane group are those of general formula (VI)

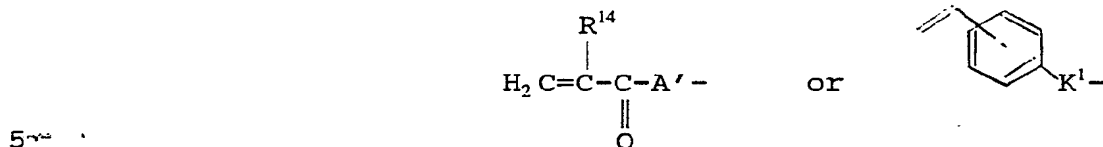


where Y^1 is an ethylenically unsaturated polymerisable group selected from

WO 93/01221

PCT/GB92/01215

- 37 -



where R^{14} is hydrogen or $\text{C}_1\text{-C}_4$ alkyl,

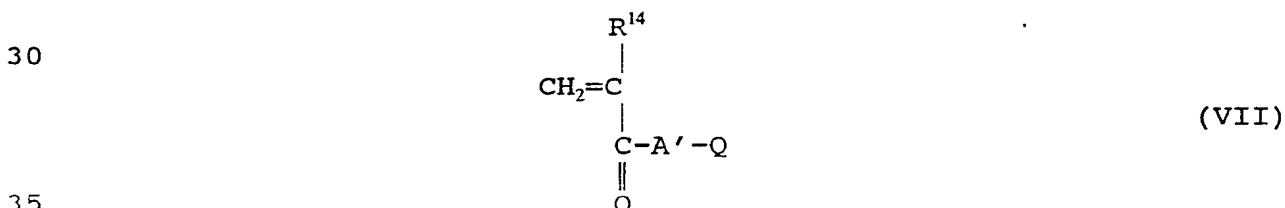
A' is $-\text{O}-$ or $-\text{NR}^{15}-$ where R^{15} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group or R^{15} is a group Q;

10 K^1 is a group $-(\text{CH}_2)_1\text{OC(O)}-$, $-(\text{CH})_1\text{C(O)}\text{O}-$,
 $-(\text{CH}_2)_1\text{OC(O)}\text{O}-$, $-(\text{CH}_2)_1\text{NR}^{16}-$, $-(\text{CH}_2)_1\text{NR}^{16}\text{C(O)}-$,
 $-(\text{CH}_2)_1\text{C(O)}\text{NR}^{16}-$, $-(\text{CH}_2)_1\text{NR}^{16}\text{C(O)}\text{O}-$, $-(\text{CH}_2)_1\text{OC(O)}\text{NR}^{16}-$,
 $-(\text{CH}_2)_1\text{NR}^{16}\text{C(O)}\text{NR}^{16}-$ (in which the groups R^{16} are the same or
different), $-(\text{CH}_2)_1\text{O}-$, $-(\text{CH}_2)_1\text{SO}_3-$, a valence bond and 1 is from
15 1 to 12 and R^{16} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group; and

Q is (a) a straight or branched alkyl, alkoxyalkyl or
(oligo-alkoxy)alkyl chain containing 6 or more, preferably 6 to
24, carbon atoms unsubstituted or substituted by one or more
fluorine atoms and optionally containing one or more carbon-
20 carbon double or triple bonds; or

(b) a siloxane group $-(\text{CR}^{16a})_2)_{qq} (\text{SiR}^{16b})_2 (\text{OSiR}^{16b})_{pp}\text{R}^{16b}$ in
which each group R^{16a} is the same or different and is hydrogen
or alkyl of 1 to 4 carbon atoms or aralkyl, for example benzyl
or phenethyl, each group R^{16b} is alkyl of 1 to 4 carbon atoms,
25 qq is from 1 to 6 and pp is from 0 to 49.

Preferred comonomers of formula (VI) bearing a group Q
include those of formula (VII) and (VIII):



WO 93/01221

PCT/GB92/01215



wherein:

R¹⁴, A', K¹ and Q are as defined in relation to formula (VI).

Preferably in the compounds of formula (VII) R¹⁴ is hydrogen methyl or ethyl, more preferably methyl so that the compound of formula (VII) is preferably an acrylic acid, methacrylic acid or ethacrylic acid derivative.

In the compounds of formula (VIII) K¹ may for instance be a valence bond. Where K¹ is a group then preferably 1 is from 1 to 6, more preferably 1, 2 or 3 and most preferably 1 is 1. When K¹ is a group -(CH₂)₁NR¹⁶-, -(CH₂)₁OC(O)NR¹⁶-, -(CH₂)₁NR¹⁶C(O)O-, -(CH₂)₁NR¹⁶C(O)-, -(CH₂)₁C(O)NR¹⁶- or -(CH₂)₁NR¹⁶C(O)NR¹⁶- then R¹⁶ is preferably hydrogen, methyl or ethyl, more preferably hydrogen.

In the compounds of formula (VIII), preferably the vinyl group is para to the group -K¹-Q.

Preferably Q is an alkyl or fluoroalkyl group optionally containing one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds. More preferably Q is:

an alkyl group of formula -(CR¹⁷₂)_mCR¹⁷₃, wherein the groups -(CR¹⁷₂)- are the same or different, and in each group -(CR¹⁷₂)- the groups R¹⁷ are the same or different and each group R¹⁷ is hydrogen, fluorine or C₁₋₄ alkyl or fluoroalkyl and m is from 5 to 23 if Q contains no fluorine atoms or from 1 to 23, preferably 5 to 23, if Q contains one or more fluorine atoms;

an alkoxyalkyl having 1 to 12 carbon atoms in each alkyl moiety; unsubstituted or substituted by one or more fluorine atoms; or

WO 93/01221

PCT/GB92/01215

- 39 -

an (oligo-alkoxyl) alkyl group of formula

$-\left[(\text{CR}^{18}_2)_n\text{O}\right]_o(\text{CR}^{18}_2)_n\text{R}^{18}$ where the groups $-(\text{CR}^{18}_2)-$ are the same or different and in each group $-(\text{CR}^{18}_2)-$ the groups R^{18} are the same or different and each group R^{18} is hydrogen, fluorine or C_{1-4} alkyl or fluoroalkyl and n is from 2 to 6, preferably 3 to 4, and o is from 1 to 12.

When Q is a group $-\left[(\text{CR}^{18}_2)_n\text{O}\right]_o(\text{CR}^{18}_2)_n\text{R}^{18}$ wherein all the groups R^{18} are hydrogen and in all the groups

$-\left[(\text{CR}^{18}_2)_n\text{O}\right]-$ n is 2 the group of formula Q is not able to form strong secondary valence interactions with hydrophobic surfaces. Whilst residues of monomers containing such a group may be included in the polymers of the invention, it is also necessary to include residues of monomers which are capable of forming such strong secondary valence interactions if such interactions are to bind a polymer to a surface. Monomers which have groups containing oligo(higher alkylene) oxide moieties can be used to provide monomers which contain oligo alkylene oxide moieties in which at least 50mol% of individual alkylene oxide units contain 3 or more carbons atoms. Thus, for instance a mixed oligo(ethylene oxide/propylene oxide) side chain could be used provided that there are more propylene oxide units than ethylene oxide units.

Where Q is an (oligo-alkoxy)-alkyl group containing residues $-\left[(\text{CR}^{18}_2)_n\text{O}\right]-$ wherein n is 2, then preferably n is 2 in no more than 50 mol% of the residues $-\left[(\text{CR}^{18}_2)_n\text{O}\right]-$.

Alternatively, Q may be a group in which one or more of the alkyl or alkylene moieties in such an alkyl, alkoxyalkyl or (oligoalkoxy) alkyl group is replaced by a corresponding alkenyl, alkynyl, alkenylene or alkynylene moiety.

WO 93/01221

PCT/GB92/01215

- 40 -

Preferred groups Q include alkyl, alkoxyalkyl and (oligo-alkoxy)alkyl groups optionally containing one or more carbon-carbon double or triple bonds of 8 or more, more preferably 10 or more, even more preferably 12 or more, for instance 14 or more, such as 16 or more carbon atoms. Such groups may contain one or more fluorine atoms and be therefore fluoroalkyl derivatives. Preferably however, such groups do not contain any fluorine atoms.

Particularly preferred groups are straight chain alkyl or fluoroalkyl groups optionally containing one or more carbon-carbon double or triple bonds.

Where Q is a siloxane group, each group $-(CR^{16a})_2-$ may be the same or different, preferably the same, and preferably each group R^{16a} is hydrogen. Preferably qq is from 2 to 4, and is most preferably 3. Each group $-(SiR^{16b})_2-$ may be the same or different, preferably the same, and preferably each group R^{16b} is methyl. Preferably pp is from 4 to 29. Preferred comonomers where Q is a siloxane group are those of formula (VII).

In one specific embodiment the group Q does not contain any ethylenic unsaturation, i.e. any carbon-carbon double or triple bonds.

Particular examples of comonomers containing an alkyl, fluoroalkyl or siloxane group include: n-dodecyl methacrylate, octadecyl methacrylate, hexadecyl methacrylate, 1H,1H,2H,2H-heptadecafluorodecyl methacrylate, p-octyl styrene, p-dodecyl styrene and monomethacryloxypropyl terminated siloxanes. n-Dodecyl methacrylate is particularly preferred.

Comonomers containing a physisorbable alkyl or

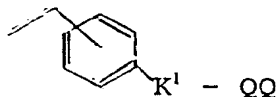
fluoroalkyl, which does not contain a carbon-carbon double or triple bond, or a siloxane group such as those of formulae (VII) and (VIII) are commercially available or may be prepared by conventional techniques using known reactions.

5 In a second specific embodiment of such comonomers, the group Q does contain ethylene unsaturation, i.e. one or more carbon-carbon double or triple bonds. Such comonomers may for example contain a vinylic, divinylic, acetylenic or diacetylenic moiety. Comonomers containing acetylenic rather
10 than vinylic unsaturation are in general preferred, especially those containing a single acetylenic group.

Comonomers which contain such an ethylenic unsaturated group are capable of providing crosslinking between linear polymer chains once the polymer is coated onto a substrate, as
15 well as binding to the substrate by physisorption. Such crosslinking may improve the stability of the coating and is typically formed by irradiation, for example with UV-or gamma-radiation. The crosslinking of such groups may be employed either alone or in addition to the use of a comonomer
20 containing a reactive group as a crosslinkable comonomer as described below.

Particularly preferred crosslinkable comonomers capable of binding to a substrate by physisorption are those of formula (VIIA) and (VIIIIA).

25



(VIIIIA)

WO 93/01221

PCT/GB92/01215

- 42 -

in which R^{14} , A' and K^1 are as hereinbefore defined and QQ is an alkynyl group containing 6 or more carbon atoms and one or two, preferably one, carbon-carbon triple bonds provided that the acetylenic moieties are not directly bonded to A' or K^1 .

5 The present invention provides, as a further feature, comonomers of formula (VIIA) and (VIIIIA).

Amongst such comonomers it is preferred that QQ is a group containing from 6 to 24 carbon atoms, preferably 8 or more, more preferably 10 or more, even more preferably 12 or
10 more, for instance 14 or more, such as 16 or more carbon atoms.

It is also preferred that the group QQ does not contain a terminal acetylenic moiety, i.e. a group $-C\equiv CH$.

A particularly preferred group QQ is 7-dodecynyl and a specific example of a compound of formula (VIIA) containing
15 such a group is dodec-7-yn-1-ol methacrylate.

The compound of formula (VIIA) and (VIIIIA) and other comonomers of formula (VII) and (VIII) containing an ethylenically unsaturated polymerisable group Q, may be prepared by analogy with known methods. Their preparation is
20 illustrated by Reference Example 5.

b) Comonomers bearing a reactive group

Preferred comonomers, which are suitable for providing binding to a hydrophilic surface having functional groups, contain a reactive group capable of covalently binding to a
25 surface and are of general formula (IX)

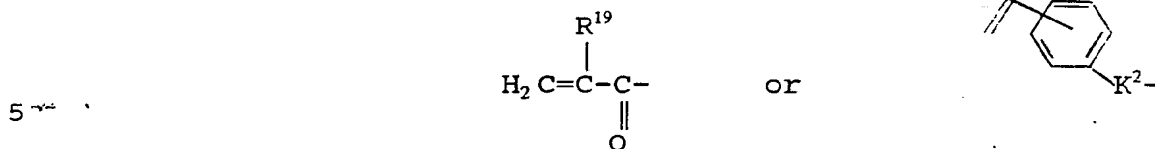


where Y^2 is an ethylenically unsaturated polymerisable group selected from

WO 93/01221

PCT/GB92/01215

- 43 -



where R^{19} is hydrogen or $\text{C}_1\text{-C}_4$ alkyl, .

K^2 is a group $-(\text{CH}_2)_q\text{OC}(\text{O})-$, $-(\text{CH})_q\text{C}(\text{O})\text{O}-$,

10 $-(\text{CH}_2)_q\text{OC}(\text{O})\text{O}-$, $-(\text{CH}_2)_q\text{NR}^{20}-$, $-(\text{CH}_2)_q\text{NR}^{20}\text{C}(\text{O})-$,

$-(\text{CH}_2)_q\text{C}(\text{O})\text{NR}^{20}-$, $-(\text{CH}_2)_q\text{NR}^{20}\text{C}(\text{O})\text{O}-$, $-(\text{CH}_2)_q\text{OC}(\text{O})\text{NR}^{20}-$,

$-(\text{CH}_2)_q\text{NR}^{20}\text{C}(\text{O})\text{NR}^{20}-$ (in which the groups R^{20} are the same or

different), $-(\text{CH}_2)_q\text{O}-$, or $-(\text{CH}_2)_q\text{SO}_3-$, or a valence bond and q is

from 1 to 12 and R^{20} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group; and

15 Q^1 is a reactive group capable of reacting to provide covalent binding to a surface.

Preferred comonomers of formula (IX) bearing a reactive group Q^1 include those of formula (X) and (XI) defined below.

The compounds of formula (X) are:

20



25

30 wherein:

R^{19} is as defined with reference to formula (X), and Q^2 is a reactive group.

Preferably in the compounds of formula (X) R^{19} is hydrogen, methyl or ethyl, more preferably methyl, so that the compound of formula (X) is preferably an acrylic acid, methacrylic acid or ethacrylic acid derivative.

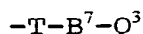
35

Preferably Q^2 is hydrogen, or more preferably $-\text{OH}$ or a group of the formula:

WO 93/01221

PCT/GB92/01215

- 44 -



where T is -O-, or -NR²¹- where R²¹ is hydrogen, C₁-C₄ alkyl
or a group -B⁷-Q³;

B⁷ is a valence bond or, more preferably, a straight or
5 branched alkylene, oxaalkylene or oligo-oxaalkylene chain; and

Q³ is a reactive group capable of reacting to provide
covalent binding to a surface such as an aldehyde group or a
silane or siloxane group containing one or more reactive
substituents such as halogen, for example chlorine, or alkoxy,
10 generally containing from 1 to 4 carbon atoms, for example
methoxy or ethoxy, or, more preferably Q³ is a hydroxyl, amino,
carboxyl, epoxy, -CHOHCH₂Hal, (in which Hal is a halogen atom
such as chlorine, bromine or iodine) succinimido, tosylate
such as 2(N-methylpyridinium) tosylate, triflate, imidazole
15 carbonyl-amino, or an optionally substituted triazine group.

Preferably B⁷ is:

an alkylene group of formula -(CR²²)_r-, wherein the groups
-(CR²²)₂- are the same or different, and in each group -(CR²²)₂-
the groups R²² are the same or different and each group R²² is
20 hydrogen or C₁₋₄ alkyl, preferably hydrogen, and r is from 1 to
12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6
carbon atoms in each alkyl moiety; or

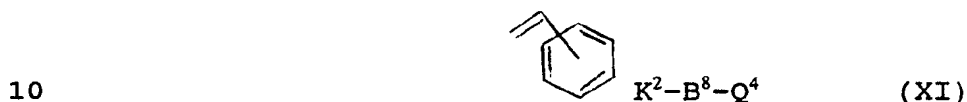
an oligo-oxaalkylene group of formula
25 -[(CR²³)₂O]_s-(CR²³)_t-, where the groups -(CR²³)₂- are the same or
different and in each group -(CR²³)₂- the groups R²³ are the same
or different and each group R²³ is hydrogen or C₁₋₄ alkyl,
preferably hydrogen, and s is from 1 to 6, preferably 2 or 3,
and t is from 1 to 11, preferably 1 to 5.

Preferred groups B^7 include alkylene, oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon atoms.

Where Q^3 is a silane or siloxy group, preferably B^7 is an alkylene group of 1 to 6, preferably 2 to 4, more preferably 3 to 5 carbon atoms.

Particular examples of the group B^7 are $-CH_2-$, $-CH_2CH_2-$ and $-(CH_2)_6-$.

The compounds of formula (XI) are:



wherein K^2 is as defined in relation to formula (IX) and;

B^8 is a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene chain and

Q^4 is a reactive group capable of reacting to provide covalent binding to a surface, for example an aldehyde group or a silane or siloxane group containing one or more reactive substituents such as halogen, for example chlorine, or alkoxy, generally containing from 1 to 4 carbon atoms, for example methoxy or ethoxy, or, more preferably, Q^4 is a hydroxyl, amino, carboxyl, epoxy, $-CHOHCH_2Hal$, (in which Hal is a halogen atom such as chlorine, bromine or iodine) succinimido, tosylate, triflate, imidazole carbonyl-amino or optionally substituted triazine group.

In the compounds of formula (XI) preferably the vinyl group is para to the group $-K^2-B^8-Q^4$.

K^2 may for instance be a valence bond. Where K^2 is a group then preferably q is from 1 to 6, more preferably 1, 2 or 3 and most preferably q is 1. When K^2 is a group $-(CH_2)_qNR^{20}-$, $-(CH_2)_qOC(O)NR^{20}-$, $-(CH_2)_qNR^{20}C(O)O-$,

WO 93/01221

PCT/GB92/01215

- 46 -

$-(CH_2)_qNR^{20}C(O)-$, $-(CH_2)_qC(O)NR^{20}-$ or $-(CH_2)_qNR^{20}C(O)NR^{20}-$ then R^{20} is preferably hydrogen, methyl or ethyl, more preferably hydrogen.

Preferably B^8 is:

an alkylene group of formula $-(CR^{24})_u-$, wherein the groups
5 $-(CR^{24})_2-$ are the same or different, and in each group $-(CR^{24})_2-$ the groups R^{24} are the same or different and each group R^{24} is hydrogen or C_{14} alkyl, preferably hydrogen, and u is from 1 to 12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6
10 carbon atoms in each alkyl moiety; or

an oligo-oxaalkylene group of formula
 $-[(CR^{25})_2O]_v(CR^{25})_w-$ where the groups $-(CR^{25})_2-$ are the same or different and in each group $-(CR^{25})_2-$ the groups R^{25} are the same or different and each group R^{25} is hydrogen or C_{14} alkyl,
15 preferably hydrogen, and v is from 1 to 6, preferably 2 or 3, and w is from 1 to 12, preferably 1 to 6.

Preferred groups B^8 include alkylene, oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon atoms. In one embodiment B^8 and K^2 contain together up to 12 carbon atoms.

20 Particular examples of comonomers bearing a reactive group include chloromethylstyrene, methacrylic acid, 2-aminoethylmethacrylate, 2,3-epoxypropyl methacrylate, 3-chloro-2-hydroxypropylmethacrylate, 2-methacryloyloxy-ethyl dichlorotriazine, 3-chloro-2-hydroxy-propylmethacrylamide and
25 glycidyl methacrylate and reactive methacrylate esters containing the group $HetC(O)O-$ in which (Het) is a heterocyclic ring, for example benzotriazole or imidazole and reactive methacrylate esters containing a group $R^{16}OC(O)-$ in which R^{16} is a succinimido or pentafluorophenyl group.

Particularly preferred comonomers bearing reactive groups are 2-aminoethyl-methacrylate and 3-chloro-2-hydroxypropylmethacrylate.

Comonomers bearing a reactive group capable of binding covalently to a surface, such as those of formula (X) or (XI), are commercially available or may be prepared by conventional techniques using known reactions.

Comonomers of formula (X), which are dichlorotriazine monomers may be prepared in known manner for example by reacting a substituted hydroxy-alkyl(alk)acrylate or aminoalkyl(alk)acrylate with trichlorotriazine in a suitable solvent and in the presence of a base.

Comonomers of formula (XI) which are reactive methacrylate esters in which the ester groups contains an imidazole group may be prepared in known manner by reacting a substituted hydroxyalkyl(alk)acrylate (e.g. 2-hydroxyethyl-(meth)acrylate), polyethylene-oxide(meth)acrylate or polypropyleneoxide (meth)acrylate with 1,1-carbonyl-diimidazole in a dry solvent. Analogous known methods may be used to prepare succinimido and pentafluorophenyl methacrylate esters of formula (X), by reaction with a reactive ester, acid halide or acid anhydride.

Where comonomers containing a reactive group are used to bind a copolymer to a surface by covalent bonding, it will be appreciated that not all of the reactive groups need necessarily bind to surface reactive groups and that groups not so bound may participate in other chemistry. Such groups may in particular provide points for the attachment of moieties such as ligands to the polymer, when coated onto a substrate.

WO 93/01221

PCT/GB92/01215

- 48 -

Comonomers containing a reactive group, such as compounds of formula (X) and (XI) may be used as comonomers containing crosslinkable groups, which react with other crosslinkable groups, rather than a monomer which bind covalently to a surface.

Where comonomers containing a reactive group are used to provide such crosslinkable groups then the crosslinkable groups and/or the copolymerisation conditions will be chosen so that they will not crosslink when the comonomers are copolymerised; thus the polymerisation product will be an uncrosslinked linear copolymer which may be subsequently crosslinked after coating the copolymer onto a surface so as to improve the stability of the coating. When such crosslinking between linear polymer chains is employed the crosslinkage may be formed either between two such crosslinkable groups or between a crosslinkable group and a non-inert group in a diluent comonomer residue (defined later). Such a crosslinkage may be formed either by direct reaction of the groups forming the crosslinkage or by reaction of these groups with a reactive bridging molecule for example a reactive gas, such as ammonia.

Residues of such comonomers may therefore be present in polymers which are designed to coat hydrophobic surfaces and containing residues of a monomer containing a group bearing a centre of permanent positive charge which is of formula (VA), (VB) or (VC) or a comonomer containing an alkyl, fluoroalkyl or siloxane group, which is of formula (VII) or (VIII). Similarly residues of such comonomers may also be present in polymers designed to bind to a surface by ionic interaction and which

contains residues of a compound of formula (XIII) or (XIV) as defined below.

Preferred reactive comonomers which are used to crosslink the comonomer, rather than provide covalent binding to the surface, are those of formula (X) or (XI) in which Q², or Q⁴ contains a crosslinkable cinnamyl, epoxy, -CHOHCH₂Hal (in which Hal is a halogen atom), methylol, silyl, an ethylenically unsaturated crosslinkable group, such as an acetylenic, diacetylenic, vinylic or divinyl group, or an acetoacetoxy or chloroalkyl sulfone, preferably chloroethyl sulphone, group.

Particular examples of comonomers bearing a group capable of crosslinking include methacrolein, cinnamyl methacrylate, 2,3-epoxypropyl methacrylate, 3-chloro-2-hydroxypropyl methacrylate, hydroxymethyl methacrylamide, 3-(trimethoxysilyl)propyl methacrylate, 2-acetoacetoxyethyl methacrylate, 3-(vinylbenzyl)-2-chloroethyl sulfone.

When a polymer of the invention, containing crosslinkable groups, is coated on a substrate the polymer is in substantially uncrosslinked form. After coating, crosslinking of crosslinkable groups may be performed to increase the strength and stability of the polymer coating.

c) Comonomers bearing an ionic group

Preferred comonomers bearing an ionic group capable of binding to a surface by ionic interaction are of general formula (XII)

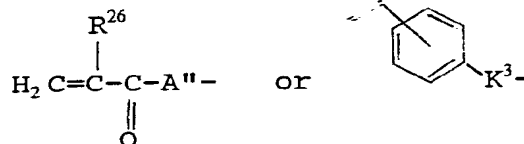


where Y² is an ethylenically unsaturated polymerisable group selected from

WO 93/01221

PCT/GB92/01215

- 50 -



5

where R^{26} is hydrogen or $\text{C}_1\text{-C}_4$ alkyl;

A'' is $-\text{O}-$ or $-\text{NR}^{27}-$, wherein R^{27} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group or R^{27} is a group $-\text{B}^9\text{-Q}^5$;

10 B^9 is a valence bond, a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group;

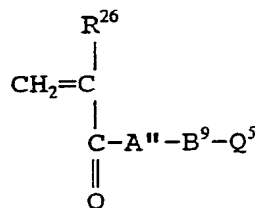
K^3 is a group $-(\text{CH}_2)_x\text{OC}(\text{O})-$, $-(\text{CH})_x\text{C}(\text{O})\text{O}-$, $-(\text{CH}_2)_x\text{OC}(\text{O})\text{O}-$, $-(\text{CH}_2)_x\text{NR}^{28}-$, $-(\text{CH}_2)_x\text{NR}^{28}\text{C}(\text{O})-$, $-(\text{CH}_2)_x\text{C}(\text{O})\text{NR}^{28}-$, $-(\text{CH}_2)_x\text{NR}^{28}\text{C}(\text{O})\text{O}-$, $-(\text{CH}_2)_x\text{OC}(\text{O})\text{NR}^{28}-$,

15 $-(\text{CH}_2)_x\text{NR}^{28}\text{C}(\text{O})\text{NR}^{28}-$ (in which the groups R^{28} are the same or different), $-(\text{CH}_2)_x\text{O}-$, $-(\text{CH}_2)_x\text{SO}_3-$, a valence bond (optionally in combination with B^9) and x is from 1 to 12 and R^{28} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group;

Q^5 is an ionic group capable of binding to a surface by
20 ionic interaction.

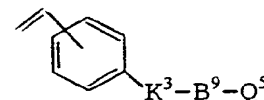
Preferred comonomers of formula (XII) are therefore those of formula (XIII) and (XIV):

25



30

(XIII)



(XIV)

35 wherein:

R^{26} , A'' , B^9 , K^3 and Q^5 are as defined in relation to formula (XII).

Preferably in the compounds of formula (XIII) R^{26} is

WO 93/01221

PCT/GB92/01215

- 51 -

hydrogen, methyl or ethyl, more preferably methyl, so that the compound of formula (XIII) is preferably an acrylic acid, methacrylic acid or ethacrylic acid derivative.

In the compounds of formula (XIV), K^3 may for instance be a valence bond. Where K^3 is a group then x is preferably from 1 to 6, more preferably 1, 2 or 3 and most preferably x is 1. When K^3 is a group $-(CH_2)_xNR^{26}-$, $-(CH_2)_xOC(O)NR^{26}-$, $-(CH_2)_xNR^{26}C(O)O-$, $-(CH_2)_xNR^{26}C(O)-$, $-(CH_2)_xC(O)NR^{26}-$ or $-(CH_2)_xNR^{26}C(O)NR^{26}-$ then R^{26} is preferably hydrogen, methyl or ethyl, more preferably hydrogen.

In the compounds of formula (XIV) preferably the vinyl group is para to the group $-K^3-B^8-Q^4$.

Preferably B^9 is:

an alkylene group of formula $-(CR^{29})_y-$, wherein the groups $-(CR^{29})_y-$ are the same or different, and in each group $-(CR^{29})_y-$ the groups R^{29} are the same or different and each group R^{29} is hydrogen or C_{1-4} alkyl, preferably hydrogen, and y is from 1 to 12, preferably 1 to 6;

an oxaalkylene group such as alkoxyalkyl having 1 to 6 carbon atoms in each alkyl moiety; or

an oligo-oxaalkylene group of formula $-[(CR^{30})_{yy}O]_{xx}(CR^{30})_{yy}-$ where the groups $-(CR^{30})_{yy}-$ are the same or different and in each group $-(CR^{30})_{yy}-$ the groups R^{30} are the same or different and each group R^{30} is hydrogen or C_{1-4} alkyl, preferably hydrogen, and yy is from 1 to 6, preferably 2 or 3, and xx is from 1 to 12, preferably 1 to 6.

Preferred groups B^9 include alkylene, oxaalkylene and oligo-oxaalkylene groups of up to 12 carbon atoms.

Particular examples of the group B^9 are $-CH_2-$,

WO 93/01221

PCT/GB92/01215

- 52 -

$-\text{CH}_2\text{CH}_2-$ and $-(\text{CH}_2)_6-$.

The group Q^5 may be either anionic or cationic depending upon the surface to be coated. Where the surface has a cationic surface charge, the group Q^5 will be anionic and may for example be a carboxylate, sulphonate, hydrogenphosphate or phosphate group. Where the surface has an anionic surface charge, the group Q^5 will be cationic and may for example be a group $-\text{NR}^{31}_3^{\oplus}$ in which each group R^{31} is the same or different, and is hydrogen or alkyl of 1 to 6 carbon atoms two of which groups R^{31} may together form a heterocyclic ring containing from 5 to 7 atoms, preferably hydrogen or methyl, a group $\text{N}^{\oplus}\text{Het}$, where Het is an unsaturated heterocyclic group such as pyridyl, substituted or unsubstituted by one or more alkyl groups of 1 to 4 carbon atoms, or a group $-\text{PR}^{32}_3^{\oplus}$ in which each group R^{32} is the same or different and is hydrogen or alkyl of 1 to 6 carbon atoms, two of which groups R^{31} may together form a heterocyclic ring containing from 5 to 7 atoms, preferably methyl.

Particular examples of comonomers bearing an ionic group include acrylic acid, methacrylic acid, 2-sulfoethyl methacrylate, 2-methacryloyloxyethyl phosphate, p-styrene sulfonic acid, 2-(methacryloyloxyethyl)trimethylammonium chloride, 3-aminopropyl methacrylamide, vinylbenzyl trimethylammonium chloride.

Comonomers bearing a group capable of binding a polymer to a surface by ionic interaction, such as those of formula (XIII) and (XIV) are commercially available or may be prepared by conventional techniques using known reactions.

Diluent Comonomers

In addition to a) the residues of monomers containing a

WO 93/01221

PCT/GB92/01215

- 53 -

group bearing a centre of permanent positive charge or b) the residues of comonomers containing a group bearing a centre of permanent positive charge and comonomers which are capable of binding to a surface, the polymers of the present invention may
5 comprise residues of a diluent comonomer.

Such diluent comonomers may be used to give the polymer the desired physical and mechanical properties. They may be of any known conventional radical polymerisable, preferably ethylenically unsaturated, type compatible with other
10 comonomer(s).

Particular examples of diluent comonomers include alkyl(alk)acrylate preferably containing 1 to 4 carbon atoms in the alkyl group of the ester moiety, such as methyl (alk)acrylate; a dialkylamino alkyl(alk)acrylate, preferably
15 containing 1 to 4 carbon atoms in each alkyl moiety of the amine and 1 to 4 carbon atoms in the alkylene chain, e.g. 2-(dimethylamino)ethyl (alk)acrylate; an alkyl (alk)acrylamide preferably containing 1 to 4 carbon atoms in the alkyl group of the amide moiety; a hydroxyalkyl (alk)acrylate preferably
20 containing from 1 to 4 carbon atoms in the hydroxyalkyl moiety, e.g. a 2-hydroxyethyl (alk)acrylate; or a vinyl monomer such as an N-vinyl lactam, preferably containing from 5 to 7 atoms in the lactam ring, for instance vinyl pyrrolidone; styrene or a styrene derivative which for example is substituted on the
25 phenyl ring by one or more alkyl groups containing from 1 to 6, preferably 1 to 4, carbon atoms, and/or by one or more halogen, such as fluorine atoms, e.g. (pentafluorophenyl)styrene.

Other suitable diluent comonomers include polyhydroxyl, for example sugar, (alk)acrylates and (alk)acrylamides in which the

WO 93/01221

PCT/GB92/01215

- 54 -

alkyl group contains from 1 to 4 carbon atoms, e.g. sugar
acrylates, methacrylates, ethacrylates, acrylamides,
methacrylamides and ethacrylamides. Suitable sugars include
glucose and sorbitol. Particularly suitable diluent comonomers
5 include methacryloyl glucose or sorbitol methacrylate.

Further diluents which may be mentioned specifically
include polymerisable alkenes, preferably of 2-4 carbon atoms,
eg. ethylene, dienes such as butadiene, alkylene anhydrides
such as maleic anhydride and cyano-substituted alkylenes, such
10 as acrylonitrile.

Diluent comonomers may be obtained by conventional known
methods.

Of the above diluent comonomers some are inert and act
simply to modify the physical and mechanical properties of
15 copolymers containing them. Others, and in particular the
hydroxyalkyl(alk)acrylates and polyhydroxyl (alk)acrylates have
a reactive role in addition to simply modifying physical and
mechanical properties. Such comonomers contain functional
groups, such as hydroxyl groups, which may react with a
20 crosslinking group or may react with reactive groups in other
molecules to attach them to the copolymer.

It will also be appreciated that alkyl(alk)acrylates
containing 6 or more carbon atoms in the alkyl group may be
regarded as either diluent comonomers or comonomers capable of
25 binding a polymer to a surface by physisorption. In particular
it should be noted that a copolymer which contains such a
diluent comonomer and a reactive comonomer capable of reacting
at a surface to provide covalent binding to a surface may be
used to coat a hydrophilic surface, the reactive comonomer

providing binding to the surface and the diluent modifying physical and mechanical properties. However, such a copolymer may also be to coat hydrophobic surfaces, in which the "diluent" monomer will act as a comonomer capable of binding to the surface by physisorption and the comonomer capable of covalent binding will act as a crosslinkable comonomer.

According to a feature of the present invention polymers of the invention may be prepared by:

a) copolymerising a comonomer containing a group bearing a centre of permanent positive charge, preferably a zwitterionic group, a comonomer containing a group capable of stably binding the polymer to a surface and optionally a diluent and/or crosslinkable comonomer; or

b) polymerising a monomer containing a group containing a group bearing a centre of permanent positive charge, preferably a zwitterionic group, and a group capable of stably binding the polymer to a surface and optionally further comonomer containing a group capable of stably binding the polymer to the surface and a diluent and/or a crosslinkable comonomer.

Any conventional technique may be used for polymerisation, typically thermal or photochemical polymerisation. Where comonomers capable of producing crosslinking in the coated polymer film are present, the polymerisation condition are set such that crosslinking does not occur during polymerisation. Thus, for example, actinic radiation would not be used to prepare a polymer containing a comonomer which can form crosslinks by exposure to actinic radiation.

For thermal polymerisation a temperature from 40 to

WO 93/01221

PCT/GB92/01215

- 56 -

100°C, typically 50 to 80°C is used. For photochemical polymerisation actinic radiation such as gamma, U.V., visible, or microwave radiation may be used. Typically U.V. radiation of wavelength 200 to 400 nm is used.

5 The polymerisation is generally performed in a reaction medium, which is for instance a solution or dispersion using as a solvent for example acetonitrile, dimethyl formamide, chloroform, dichloromethane, ethyl acetate, dimethyl sulphoxide, dioxan, benzene, toluene, tetrahydrofuran, or where
10 the polymer does not contain groups which react with protic solvents, water or an alkanol containing from 1 to 4 carbon atoms, e.g. methanol, ethanol or propan-2-ol. Alternatively, a mixture of any of the above solvents may be used.

The polymerisation may be carried out in the presence of
15 one or more polymerisation initiators, such as benzoyl peroxide, 2,2'-azo-bis(2-methylpropionitrile) or benzoin methyl ether. Other polymerisation initiators which may be used are disclosed in "Polymer Handbook", 3rd edition, Ed. J. Brandrup and E.H. Immergut, Pub. Wiley-Interscience, New York, 1989.

20 Generally the copolymerisation is performed for 1 to 72 hours, preferably 8 to 48, for instance 16 to 24 hours, and under an inert atmosphere of for example nitrogen or argon. The polymer is generally purified by dialysis, precipitation in a non-solvent (e.g. diethyl ether or acetone) or ultrafiltration.
25 The resulting polymer is generally dried under vacuum, eg. for 5 to 72 hours and has a molecular weight from 10,000 to 10 million, preferably from 20,000 to 1 million.

The precise proportion and nature of the various comonomers used to prepare a copolymer according to the present

WO 93/01221

PCT/GB92/01215

- 57 -

invention comprising residues of a comonomer containing a group bearing a centre of permanent positive charge and a comonomer containing a group capable of stably binding the polymer to a surface may be adjusted to provide a copolymer which is particularly suitable for coating a particular surface. Thus the proportion of comonomer containing a group capable of stably binding the polymer to a surface may be adapted to provide efficient physisorption at a particular hydrophobic surface, to correspond to the number of functional groups at a particular surface or to provide efficient binding by ionic interaction with a particular surface. Similarly the proportion of the comonomer containing a group bearing a centre of permanent positive charge and of diluent and/or crosslinkable comonomer may be adapted to provide the desired biocompatibility and physical and mechanical properties. It will be appreciated that to obtain the desired combination of properties more than one type of comonomer containing a group bearing a centre of permanent positive charge, comonomer containing a group capable of stably binding the polymer to a surface or crosslinkable and/or diluent comonomer may be used.

Similarly, in polymers comprising residues of a monomer containing a group bearing a centre of permanent positive charge and a group capable of stably binding the polymer to a surface, the nature of these groups may be adjusted to provide the desired biocompatibility and efficient binding at a particular surface, as well as desired physical and mechanical properties. Where, in addition, a diluent and/or crosslinkable comonomer is used the nature of the diluent and/or crosslinkable comonomer and the proportions of the comonomers

WO 93/01221

PCT/GB92/01215

- 58 -

may be likewise adjusted. It will again be appreciated that to obtain the desired combination of properties more than one type of monomer containing a group bearing a centre of permanent positive charge and a group capable of stably binding the polymer to a surface and/or more than one type of crosslinkable and/or diluent comonomer may be used.

The monomer composition which is subjected to polymerisation to provide a polymer according to the invention comprises a minimum of 0.01%, preferably 1%, more preferably 5% by weight of monomer or monomers containing a group bearing a centre of permanent positive charge and a maximum of 99.9%, preferably 99%, more preferably 95% by weight of other monomer or monomers. Such other monomer or monomers may be a monomer or monomers containing a group capable of stably binding the polymer to a surface, a diluent monomer or monomers and/or a crosslinkable monomer or monomers.

The monomer composition further comprises a minimum of 0.01%, preferably 1%, more preferably 5% by weight of monomer or monomers containing a group capable of stably binding the polymer to a surface and a maximum of 99.9%, preferably 99%, more preferably 95% by weight of other monomer or monomers. Such other monomer or monomers may be a monomer or monomers containing a group bearing a centre of permanent positive charge, a diluent monomer or monomers and/or a crosslinkable monomer or monomers.

It will be appreciated that where at least some of the monomer or monomers containing a group bearing a centre of permanent positive charge also contains a group capable of stably binding the polymer to a surface, at least a proportion

WO 93/01221

PCT/GB92/01215

- 59 -

of the content of both these groups is provided by the same monomer. In such a case the polymer may be a homopolymer of a monomer containing both these groups.

Where the polymer is to bind to a surface by
5 physisorption then preferably the monomer composition comprises no more than 95%, more preferably no more than 90% and even more preferably no more than 80% by weight of monomer or monomers containing an alkyl, fluoroalkyl or siloxane group which is capable of binding the polymer to a surface by
10 physisorption and which does not also contain a group bearing a centre of permanent positive charge, the balance of the composition being monomer or monomers containing a group bearing a centre of permanent positive charge, diluent monomer or monomers and/or crosslinkable monomer or monomers. Such a
15 composition typically comprises up to 50% by weight of diluent comonomer or comonomers. Where diluent comonomer is present, it preferably comprises at least 1%, more preferably 5%, by weight of the total comonomer composition. Where present, crosslinkable comonomer or comonomers generally comprise from
20 0.1% to 20% by weight of the total comonomer composition.

Where different comonomers are used to provide the centre of permanent positive charge and the physisorption, then preferably the molar ratio in the copolymer of comonomer residues bearing a centre of permanent positive charge to
25 comonomer residues containing an alkyl, fluoroalkyl or siloxane group capable of binding the polymer to a surface by physisorption is from 5:95 to 80:20, more preferably 10:90 to 50:50. In addition the copolymer preferably comprises from 5% to 50%, more preferably 10% to 25%, by mole residues of diluent

WO 93/01221

PCT/GB92/01215

- 60 -

monomer and/or from 0.1 to 20%, more preferably 1% to 10%, by mole residues of crosslinkable comonomer, provided that where residues of both diluent and crosslinkable comonomer are present, they do not exceed in combination 50%, preferably 35% by mole.

Where the polymer is to bind covalently to a surface then preferably the monomer composition comprises no more than 25%, more preferably up to 20% and even more preferably up to 15% by weight of monomer or monomers containing a group capable of binding the polymer to a surface covalently and which does not also contain a group bearing a centre of permanent positive charge; the balance of the composition being monomer or monomers containing a group bearing a centre of permanent positive charge, and optionally diluent monomer or monomers. Such a composition typically comprises up to 95%, preferably to 90%, by weight of diluent comonomer or comonomers. Where diluent comonomer is present, it preferably comprises at least 5%, more preferably 10%, by weight of the total comonomer composition.

Preferably the molar ratio in the copolymer of comonomer residues bearing a centre of permanent positive charge to comonomer residues containing a reactive group capable of binding the polymer to a surface by covalent bonding is from 5:95 to 95:5, more preferably 50:50 to 90:10. In addition, the copolymer preferably comprises from 5% to 50%, more preferably 10% to 25%, by mole residues of diluent monomer and/or from 0.1% to 20%, more preferably 1% to 10%, by mole residues of crosslinkable comonomer, provided that where residues of both diluent and crosslinkable comonomer are present, they do not

WO 93/01221

PCT/GB92/01215

- 61 -

exceed in combination 50%, preferably 35% by mole.

Where the polymer is to bind to a surface by ionic interaction, then preferably the molar ratio in the copolymer of comonomer residues bearing a centre of permanent positive charge to comonomer residues containing an ionic group capable of binding the polymer to a surface by ionic interactions is from 5:95 to 95:5, more preferably 50:50 to 90:10. In addition, the copolymer preferably comprises from 5% to 50%, more preferably 10% to 25%, by mole residues of diluent monomer and/or from 0.1% to 20%, more preferably 1% to 10%, by mole residues of crosslinkable comonomer, provided that where residues of both diluent and crosslinkable comonomer are present, they do not exceed in combination 50%, preferably 35% by mole.

In addition the monomer or comonomer composition may comprise further components such as a polymerisation initiator, chain transfer agent, acid, base, surfactant, emulsifier or catalyst of conventional type each in an amount from 0.1% to 5%, typically from 0.2% to 3% and preferably about 0.5%, by weight each relative to the total weight of the monomers.

As a further feature the present invention provides a process for biocompatibilising a surface which comprises coating the surface with a polymer according to the present invention. Various types of surfaces may be coated depending upon the nature of the groups in the polymer capable of binding it to the surface.

Polymers containing residues of monomers containing alkyl, fluoroalkyl or siloxane groups capable of binding the polymer to a surface by physisorption are particularly suitable

WO 93/01221

PCT/GB92/01215

- 62 -

for coating hydrophobic surfaces, e.g. polyethylene, polypropylene and polytetrafluoroethylene (PTFE) surfaces; fluorine containing polymers of the invention being particularly suited to coating PTFE surfaces.

5 Hydrophilic surfaces may be rendered hydrophobic and suitable for coating with such polymers by known methods (see for example "Chemical Reactions of Polymers" Ed. E.M. Fettes, 1964, Interscience, London).

Treatment with such a polymer is generally carried out by coating the surface with a solution, dispersion (including a microdispersion) of the polymer, generally in an alcoholic, aqueous, organic or halogenated solvent or a mixture thereof, e.g. methanol, ethanol, dichloromethane or freon. The treatment is generally carried out at ambient or elevated temperature, such as from 5 to 60°C.

In one specific embodiment of the invention, the copolymer is coated onto the substrate in the form of a microdispersion for example a microemulsion.

After coating the polymer may be crosslinked if it contains the residues of crosslinkable comonomer by known method for crosslinking the specific crosslinkable groups which are present. Crosslinking may, for instance, be introduced thermally, using actinic radiation, using reactive gases for example ammonia by changing the pH, using difunctional additives or by using activation chemistries for example by known methods as described in "Methods in Enzymology, volume 135, Immobilised Enzymes and Cells, part B", Ed. K. Mosbach, Academic Press Inc, New York, 1987. This activation may be performed on the dry coating, in the cases of thermal radiation

WO 93/01221

PCT/GB92/01215

- 63 -

or gas treatment. Alternatively for cases where the pH needs to be changed or additives need to be included, activation may be performed on the coated material in a solution which does not remove the coating.

5 Surfaces having functional groups such as hydroxyl, carboxyl or amino groups are particularly suitable for treatment with polymers according to the invention comprising residues of monomer containing a group capable of binding the polymer to a surface covalently.

10 Where necessary the surface of the substrate may be functionalised prior to treatment. For surfaces which do not have functional groups it is necessary to introduce these groups at the surface before treatment with the polymer. This can be effected by known etching or derivatising techniques,
15 such as plasma discharge, which introduce the appropriate surface functionality (see for example "Chemical Reactions of Polymers" Ed. E.M. Fettes, 1964, Interscience, London).

In certain cases it is also necessary to activate functional groups at the surface of the substrate and/or the
20 reactive groups of the polymer of the invention. This may be achieved by known means using a known activating agent for example a carbodiimide such as 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide. Other suitable activating agents are disclosed in "Methods in Enzymology", supra. It will be
25 appreciated that corresponding methods of activation of groups on a polymer may also be used to attach moieties, such as ligands to the polymer when coated on a substrate.

Treatment with such a polymer is generally carried out by treating the surface with a solution of the polymer, generally

WO 93/01221

PCT/GB92/01215

- 64 -

an alcoholic, aqueous alcoholic or aqueous solution. The treatment is generally carried out at a temperature from -5 to ~50°C, for from 0.1 to 24 hours and at a pH from 2 to 13.

Surfaces having ionic groups such as carboxyl, sulphonate, phosphate, ammonium or phosphonium groups are particularly suitable for treatment with polymers according to the invention comprising residues of monomer containing a group capable of binding the polymer to ionic interaction.

Where necessary the surface of the substrate may be functionalised prior to treatment. For surfaces which do not have ionic groups it is necessary to introduce these groups at the surface before treatment with the polymer. This can be effected by known etching or derivatising techniques, such as plasma discharge, which introduce the appropriate surface functionality (see for example "Chemical Reactions of Polymers" Ed. E.M. Fettes, 1964, Interscience, London)

Treatment with such a polymer is generally carried out by treating the surface with a solution of the polymer, generally an alcoholic, aqueous alcoholic or aqueous solution. Treatment is generally carried out at a temperature from -5 to 50°C, for from 0.1 to 24 hours and at a pH from 2 to 13.

Materials may be coated with polymers of the invention by known techniques, such as dip-coating, spray-coating, web-coating or spin coating.

Materials having surfaces coated according to the present invention can be used as a construction material for implants or prostheses for the human or animal body, particularly where these implants or prostheses are to come into direct physical contact with blood and where biocompatibility and particularly

WO 93/01221

PCT/GB92/01215

- 65 -

haemocompatibility are required e.g. in heart valves. They can also be used in the construction of membranes and other devices that are to be brought into contact with blood or other body fluids on an extra-corporeal basis, for example in heart-lung machines or artificial kidneys.

Additionally the polymers of the invention can be used to coat materials employed in down stream processing applications e.g. separation membranes and process equipment and tubing. In particular the materials of the invention can be used to modify the surface properties of biofiltration membranes in bioreactors and fermentation systems, where the membranes come into direct contact with complex biological solutions containing e.g. proteins, polysaccharides, fats and even whole cells. The polymers of the invention are particularly useful in reducing membrane fouling by the components of a process solution.

When the polymers of the present invention are used to coat the surface of a material which is then used in the construction coat of finished devices, it may be necessary to take precautionary steps to ensure that the coated surface is not damaged and the effectiveness of the treatment reduced before the finished device is produced.

In addition, the polymers of the present invention can be used to coat finished implants, prostheses, membranes, catheters, contact lenses, intraocular lenses, and other devices which are coated with a polymer according to the present invention to impart biocompatibility to the article.

The invention thus also provides a finished device comprising a surface having a coating thereon of a polymer of

WO 93/01221

PCT/GB92/01215

- 66 -

the present invention.

Figure 1 compares scanning electron micrographs of a polyimide sheet treated and untreated with a copolymer of the invention and then contacted with blood. Figure 1.(a) shows a scanning electron micrograph (1200x) of an unsubbed, untreated poly(imide) sheet. Figure 1.(b) shows a scanning electron micrograph (900x) of a poly(acrylic acid) subbed poly(imide) sheet treated with poly (2(methacryloyloxyethyl)-2(trimethylammonium)ethyl phosphate-co-2-aminomethacrylate) (9:1), in accordance with Example 8.

The present invention will now be further illustrated by the following Examples:

Examples

The following assays have been used to evaluate coatings of polymers according to the present invention.

Protein adsorption using an enzyme immunoassay

The assay determines adsorption of human fibrinogen at a surface. This protein is representative of protein which is typically adsorbed at a surface. The assay can be readily modified to determine the adsorption of other proteins.

Discs (7mm in diameter) of untreated material (as controls) and material treated with polymer as described below, were prepared and washed with phosphate buffered saline (PBS) for at least 10 minutes in the wells of microplates. The samples were incubated with human plasma (300 μ l) for 10 minutes and then washed with PBS three times. Each of the test samples and each of the control samples were treated with human fibrinogen-specific antibody (300 μ l) for 30 minutes and again

WO 93/01221

PCT/GB92/01215

- 67 -

washed with PBS three times. As a control for non-specific binding of antibody to the samples, each sample was also incubated with non-specific antibody (300 μ l) for 30 minutes. A conjugate of horseradish peroxidase and a second antibody specific to the first antibody (300 μ l) was added to both the test samples and the controls and incubated for 30 minutes before washing. Each of the test samples and the controls were transferred to new microplates and a solution of 2,2'-azino-bis(3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) in phosphate-citrate buffer (300 μ l, 0.6mg/ml) added, the reaction was allowed to proceed for 10 minutes. At this time an aliquot of the mixture (200 μ l) was removed and added to a solution of citric acid and sodium azide in distilled water (20 μ l, 0.21g/ml and 2mg/ml respectively). The optical density of the solutions was measured using a Techgen automated plate reader at 650nm using the ABTS solution as blank.

In an alternative procedure, rather than using ABTS, each of the samples was transferred to wells of new microplates and a solution of o-phenylene diamine (OPD) in phosphate-citrate buffer (300 μ l, 0.4mg/ml) added, and the reaction was allowed to proceed for 10 minutes. At this time an aliquot of the mixture (200 μ l) was removed from each well and the optical density of the solutions was measured using a Techgen automated plate reader at 450nm using the OPD solution as blank.

25

Activated Platelet Study

Blood was collected from a healthy adult volunteer using the double syringe method where the first 5ml of blood is discarded. The blood was collected into tri-sodium citrate

WO 93/01221

PCT/GB92/01215

- 68 -

(32g/l) in the proportion of 9 volumes to 1 volume citrate in plastic tubes. The samples were kept at room temperature on a spiral mixer until used.

Discs (7mm in diameter) of untreated material as controls and material treated with polymers as described below were prepared and placed into the wells of a microplate. The samples were incubated with whole fresh citrated blood (200 μ l) on a rotary mixer for 30 minutes before washing in PBS four times. Platelet activation was measured by a proprietary assay [Lindon, J.N. et al., Blood, 68, 355 (1986)] and British Patent Application No. 91-25721.2].

In an alternative procedure half of the test replicates were incubated with citrated blood (200 μ l) and the remainder were incubated with EDTA-treated blood on a phase shaker for 30 minutes before washing in PBS four times. Platelet activation was measured in a manner similar to that described above for detection of proteins by enzyme immunoassay using antibodies against GMP140 to detect the presence of this platelet activation marker on the surface of biomaterials. In the presence of EDTA, which extracts calcium from inside platelets, activation is inhibited, so that incubation with EDTA-treated blood acts as a non-specific control for activation, obviating the need for incubation in non-specific antibody.

C-Reactive protein (CRP) binding assay

C reactive protein is a protein which binds specifically to isolated ammonium phosphate esters groups e.g. phosphoryl choline groups which are attached to a surface.

Discs (7mm in diameter) of untreated material and material treated with polymer as described below, were prepared and washed with HEPES-buffered saline (HBS) for a least 10

minutes in the wells of microplates. The samples were incubated in quadruplet for 45 minutes in a protein solution consisting of bovine serum albumin (BSA) (40 mg/ml) and CRP (0.012 mg/ml) in HBS and containing calcium chloride (1mM). In parallel, 5 identical samples (both coated and uncoated) were incubated either in BSA/Ca²⁺ solution in the absence of CRP, in BSA/CRP/Ca²⁺ solution in the presence of soluble phosphoryl choline (1.5 mg/ml) or in BSA/CRP solution containing EDTA (20mM) rather than calcium chloride.

10 After incubation, all the samples were washed in phosphate buffered saline (PBS) three times and then incubated for 1 hour in 300μl of a 1:100 dilution of commercially available anti-CRP antibody conjugated with horseradish peroxidase. The samples were washed three times in PBS as 15 before and transferred to new microplates. A solution of o-phenylene diamine (OPD, 0.4 mg/ml) in phosphate-citrate buffer was added and the reaction allowed to proceed for ten minutes. At this time an aliquot of the mixture (200μl) in each of the wells was transferred to a new well, and the optical density of 20 the solutions measured using a Techgen automated plate-reader at 450 nm using the OPD solution as a blank.

A positive control containing isolated phosphoryl choline groups may be provided using beaded agarose immobilised with p-aminophenylphosphoryl choline. The specificity of CRP binding 25 may be demonstrated by inhibition by phosphoryl choline and dependance upon the presence of calcium.

Example 1

Preparation of poly(2(methacryloyloxyethyl)-

2'(trimethylammonium)ethyl phosphate inner salt -co-n-dodecyl

WO 93/01221

PCT/GB92/01215

- 70 -

methacrylate (1:2)

-- 2-(Methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate inner salt (5.0g, 0.0170 mole) and n-dodecylmethacrylate
5 (8.55g, 0.0340 mole) were dissolved in methanol/THF (140ml, 5:9). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes. 2,2'-Azo-bis(2-methylpropionitrile) (0.17g, 1.02 mmole) was added and the flow of nitrogen was reduced to 10ml/min, the temperature was raised
10 to 60°C. This temperature and nitrogen flow rate were maintained for 16 hours.

The mixture was allowed to cool and vacuum filtered. The filtrate was collected and the polymer precipitated by dropwise addition to acetone (1.2l).

15 The polymer was isolated by filtration under vacuum under a nitrogen atmosphere and finally dried under reduced pressure overnight at room temperature. The resulting polymer (9.5g, 70%) was a fine white powder.

In an alternative procedure, 2-(methacryloyloxyethyl)-
20 2'-(trimethylammonium)ethyl phosphate inner salt (12.06g, 0.0409 mole) and n-dodecyl methacrylate (20.52g, 0.0808 mole) were dissolved in propan-2-ol (215ml) and ethyl acetate (85ml). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes, 2,2'-azo-bis(2-
25 methylpropionitrile) (0.0645g, 0.39mmole) was added and the flow of nitrogen was reduced to 10ml/min, the reaction temperature was raised to 60°C. This temperature and nitrogen flow rate were maintained for 40 hours.

WO 93/01221

PCT/GB92/01215

- 71 -

The mixture was allowed to cool and vacuum filtered. The filtrate was evaporated to dryness using a rotary evaporator and dissolved in dichloromethane (120ml) and methanol (10ml). The polymer was isolated from this mixture by precipitation in acetone (2500ml), vacuum filtration and drying. The polymer was redissolved in dichloromethane (100ml) and methanol (30ml) and isolated as described above.

The resulting polymer, obtained in 70-80% yield was a white powder.

10

NMR(200MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-3.8 (b), 3.3 (s), 1.8-2.2 (b), 1.5-1.8 (b), 1.2-1.5 (s), 0.8-1.0 (s)

15 IR(cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246, 1156, 1089, 968, 788.

Elemental Analysis

20	theory	C 64.5,	H 9.9,	N 1.8,	P 3.9
	actual	C 59.0,	H 10.0,	N 1.8,	P 3.9

The polymer had a relative viscosity in ethanol: chloroform (50:50) at 25°C of 1.13 ± 0.02 (when prepared using methanol: THF as solvent) and 1.26 ± 0.02 (when prepared using propan-2-ol: ethylacetate as solvent).

Example 2

The coating of poly(ethylene) ribbon with

WO 93/01221

PCT/GB92/01215

- 72 -

poly(2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl
phosphate inner salt -co- n-dodecyl methacrylate (1:2)

Poly(ethylene) ribbon was washed with ethanol and allowed to
5 dry in the air. The poly(2(methacryloyloxyethyl)-
2'(trimethylammonium)ethyl phosphate inner salt -co- n-dodecyl
methacrylate) (1:2) (50mg) was dissolved in ethanol/chloroform
(5ml, 40:1) and the poly(ethylene) coated by a one stage
mechanical dip-coating procedure drawing the ribbon through the
10 solution slowly. The coated ribbon was allowed to dry in a
dust free atmosphere at room temperature.

The treated poly(ethylene) showed a 65% reduction in
protein adsorption as compared to the untreated material and a
83% reduction in platelet activation (determined using the
15 assay of Lindon et al) as compared to the untreated material.

In the C-reactive protein binding assay, no binding of
CRP was observed to the treated poly(ethylene). In contrast,
CRP binding was observed for a positive control. The
specificity of this CRP binding was demonstrated by the fact
20 that it was inhibited by phosphoryl choline and dependence upon
the presence of calcium.

According to an alternative procedure, polyethylene
ribbon was washed in propan-2-ol and coated with the copolymer
dissolved in propan-2-ol (1g in 100ml) at 40°C using an
25 otherwise analogous manner.

Example 3

In an analogous manner to that described in Example 2
steel and PVC substrates were coated with

WO 93/01221

PCT/GB92/01215

- 73 -

poly(2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl
phosphate inner salt -co-n-dodecyl methacrylate (1:2).

The treated steel samples showed a reduction in protein
adsorption of over 80% compared to untreated samples and the
5 treated PVC samples showed a reduction in protein adsorption of
over 70% compared to untreated samples as measured by the
enzyme immunoassay described above. In a further determination
a sample of stainless steel coated with the polymer showed a
reduction, compared to untreated material, in protein
10 adsorption of 84% as determined by the enzyme immunoassay
technique and a reduction of 95% in platelet activation as
determined by the platelet activation assay described above
using anti-GMP140. A further sample of PVC coated with the
polymer showed a reduction of 87% in protein adsorption and a
15 reduction of 100% in platelet activation, compared to untreated
material, using the same assay techniques.

Example 4

Preparation of poly(2(methacryloyloxyethyl)-
20 2'(trimethylammonium)ethyl phosphate inner salt-co-n-dodecyl
methacrylate (1:4)

2(Methacryloyloxyethyl)-2'(trimethylammonium)ethyl
phosphate inner salt (10.00g, 0.0339 mole) and n-dodecyl
methacrylate (35.56g, 0.1400 mole) were dissolved in propan-2-
25 ol (200ml) and ethyl acetate (200ml). The solution was stirred
(250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30
minutes. 2,2'-azo-bis-(2-methylpropionitrile) (0.0886g,
0.54mmole) was added and the flow of nitrogen was reduced to
10ml/min, the reaction temperature was raised to 60°C. This

WO 93/01221

PCT/GB92/01215

- 74 -

temperature and nitrogen flow rate were maintained for 40 hours.

The mixture was allowed to cool and vacuum filtered. The filtrate was evaporated to dryness using a rotary evaporator and dissolved in dichloromethane (130ml). The polymer was isolated from this mixture by precipitation in acetone (2500ml), vacuum filtration and drying. The polymer was redissolved in dichloromethane (120ml) and methanol (10ml) isolated as before.

The resulting polymer, obtained in 70-80% yield was a white solid.

NMR(200MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-3.8 (b), 3.3 (s), 1.8-2.2 (b), 1.5-1.8 (b), 1.2-1.5 (s), 0.8-1.0 (s)

IR(cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246, 1156, 1089, 968, 788.

Elemental Analysis:

theory	C: 68.9,	H 10.5,	N 1.1,	P 2.4
actual	C: 65.5,	H 10.8,	N 1.1,	P 2.4

The polymer had a relative viscosity in ethanol: chloroform (50:50) at 25°C of 1.26 ± 0.02.

Samples of polyethylene, steel and PVC were coated using the methods described in Examples 2 and 3. Using the enzyme

immunoassay for protein adsorption, a greater than 80% reduction in protein adsorption on steel and greater than 70% reduction in protein adsorption on PVC were obtained.

In a further determination, a sample of stainless steel coated with the polymer showed a reduction of 80% in protein adsorption (determined by the enzyme immunoassay described above) and a reduction of 95% in platelet activation (determined by the platelet activation assay described above using anti-GMP 140), compound to untreated material. A sample of PVC coated with the polymer showed a reduction of 70% in protein adsorption and 100% in platelet activation compound to untreated material using the same assay techniques.

Example 5

15 Preparation of poly(2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt-co-1H, 1H, 2H, 2H, heptadecafluorodecyl methacrylate (2:1)

2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt (1.0g, 0.0034 mole) and 1H, 1H, 2H, 2H-heptadecafluorodecyl methacrylate (0.90g, 0.0017 mole) were dissolved in methanol (10ml) and tetrahydrofuran (10ml). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes. 2,2'-azo-bis(2-methylpropionitrile) (0.0167g, 0.10mmole) was added and the flow of nitrogen was reduced to 10ml/min, the reaction temperature was raised to 60°C. This temperature and nitrogen flow rate were maintained for 16 hours.

The mixture was allowed to cool and vacuum filtered. The filtrate was evaporated to dryness using a rotary evaporator

WO 93/01221

PCT/GB92/01215

- 76 -

and dissolved in dichloromethane (10ml) and methanol (10ml). The polymer was isolated from this mixture by precipitation in acetone (500ml), vacuum filtration and drying. The polymer was redissolved in dichloromethane (10ml) and methanol (10ml) and isolated as before. The resulting polymer, obtained in 70-80% yield was a white powder.

NMR(200MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-3.8 (b), 3.3 (s), 2.4-2.7(b), 2.2-1.7(b), 1.2-1.0(b), 0.8-1.0 (b)

The polymer was used to coat a polyvinylidene fluoride microfiltration membrane. The resulting coated membrane showed very little flux decline during processing with bovine serum albumin (BSA) indicating very little protein fouling. The flux change for the treated membrane was from 6000 to 5000 l/m²/hr compared to the flux change for the untreated membrane which was from 5000 to 500 l/m²/hr. Both measurements were taken over a two hour period.

20 Example 6

Preparation of poly(2(methacryloyloxyethyl)-2'-trimethylammonium)ethyl phosphate inner salt-co-n-hexadecyl methacrylate (1:2)

2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt (2.00g, 6.78 mmole) and n-hexadecyl methacrylate (4.21g, 0.0136 mole) were dissolved in propan-2-ol (35.5ml) and ethyl acetate (14.5ml). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes. 2,2'-azo-bis(2-methylpropionitrile) (0.0168g,

WO 93/01221

PCT/GB92/01215

- 77 -

0.10mmole) was added and the flow of nitrogen was reduced to 10ml/min, the reaction temperature was raised to 60°C. This temperature and nitrogen flow rate were maintained for 40 hours.

5 The mixture was allowed to cool and vacuum filtered. The filtrate was evaporated to dryness using a rotary evaporator and dissolved in dichloromethane (10ml) and methanol (10ml). The polymer was isolated from this mixture by precipitation in acetone (700ml), vacuum filtration and drying. The polymer was
10 redissolved in dichloromethane (10ml) and methanol (10ml) isolated as described above. The resulting polymer, obtained in 40-60% yield was a white solid.

NMR(200MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-
15 3.8 (b), 3.3 (s), 1.8-2.2 (b), 1.5-1.8 (b), 1.2-1.5 (s), 0.8-1.0 (s)

IR(cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246, 1156, 1089, 968, 788.

20

Example 7

Preparation of poly(2(methacryloyloxyethyl)-2(trimethylammonium)ethyl phosphate inner salt -co- 2-aminoethylmethacrylate) (9:1)

25 2(Methacryloyloxyethyl)-2(trimethylammonium)ethyl phosphate inner salt (9.96g, 0.0335 mole) was dissolved in methanol (115ml). Water (10ml) was added followed by the addition of 2-aminoethylmethacrylate (0.5571g, 0.0034 mole). The solution was stirred (250 rpm) at 22°C under a stream of

WO 93/01221

PCT/GB92/01215

- 78 -

nitrogen (70ml/min) for 30 minutes. 2,2'Azo-bis(2-methylpropionitrile) 0.12g, 0.73mmole) was added and the flow of nitrogen was reduced to 9ml/min, the temperature was raised to 60°C. The temperature and nitrogen flow rate were maintained for 16 hours.

The mixture was allowed to cool and transferred to centrifuge tubes. The samples were centrifuged for 30 minutes at 4000 rpm. The samples were combined and the polymer precipitated by dropwise addition to acetone (800ml). The acetone was decanted from the polymer and the polymer washed with acetone (200ml). The polymer was isolated by vacuum filtration under a nitrogen atmosphere and finally dried in vacuo overnight at room temperature.

IR (cm⁻¹;KBr disc) 3435, 2929, 2096, 1732, 1628, 1245, 1166, 1089, 970.

Example 8

Treatment of poly(acrylic acid) subbed poly(imide) sheets with poly(2(methacryloyloxyethyl)-2(trimethylammonium) ethyl phosphate inner salt -co- 2-aminoethylmethacrylate) (9:1).

Poly(imide) samples were placed in the plasma chamber of a plasma barrel etcher and evacuated with a pump down to a pressure of 0.001mbar. Oxygen was then allowed to flow into the reactor. The plasma was started with 90W forward power and nearly 0W backward. The pressure was approximately 0.7mbar. The plasma was turned on for 5 minutes, then the radio frequency generator (13.56MHz) was switched off at the same time as the flow of oxygen stopped. The pressure was allowed to drop and the valve of the flask with acrylic acid was opened

to let the monomer flow into the chamber (100% acrylic acid). The vacuum was decreased to 0.3mbar. The high frequency generator was then started with 30W forward power and 0W backward power and the polymerisation carried out for 20 minutes. After switching off the high frequency generator and closing the valve to the acrylic acid, the chamber was evaporated again for another 5 minutes to remove all of the excess monomer.

The poly(acrylic acid) subbed poly(imide) was cut into 4 x 1.5cm² pieces and washed with distilled water. The squares were then added to a 1.25% solution (6.3ml) of poly(2(methacryloyloxyethyl)-2(trimethylammonium) ethyl phosphate inner salt -co- 2-aminoethylmethacrylate (9:1). 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide (20g) was then dissolved in the solution and the pH then adjusted to 5.0 using hydrochloric acid (0.5M). After 1 hour the samples were removed, washed with distilled water and allowed to dry.

Visualisation of platelet activation on a surface

Blood was collected from a healthy adult volunteer using the double syringe method where the first 5ml of blood is discarded. The blood was collected into tri-sodium citrate (32g/l) in the proportion of 9 volumes of blood to 1 volume citrate in plastic tubes. The samples were kept at room temperature on a spiral mixer until used.

1cm² samples of poly(2(methacryloyloxyethyl)-2-(trimethylammonium)ethyl phosphate inner salt -co- 2-aminoethylmethacrylate) (9:1) coated poly(imide) as prepared above and of uncoated poly(imide) as a comparison were placed into 1ml of the fresh citrated blood and incubated for 30

WO 93/01221

PCT/GB92/01215

- 80 -

minutes on a spiral mixer at room temperature. The samples were then washed in phosphate buffered saline (PBS, pH 7.4) prior to fixing in an aliquot of the following solution for 30 minutes.

5

2ml 25% w/v glutaraldehyde
83ml 0.15M PBS (pH 7.4)
15ml Saturated picric acid.

10 Picric acid increases the preservation of lipid-associated protein. The samples were again washed in PBS and then dehydrated using 70% and 100% methanol followed by 100% acetone prior to drying in air. Finally samples were sputter-coated with a platinum target (20mAmps for 6x30 seconds) and
15 observed at appropriate magnifications using a scanning electron microscope.

No platelet activation was seen on the coated poly(imide) samples whereas gross adhesion activation and aggregation were seen on the uncoated sample (see Fig.1). The presence of the
20 polymer on the surface was confirmed by the use of X-ray photoelectron spectroscopy (XPS). It can thus be seen that treatment of polyamide by first coating with a subbing layer of acrylic acid to render the surface reactive, and then coating with a copolymer according to the present invention
25 substantially removed the haemostatic reaction to the polyamide.

Example 9

Preparation of poly(2(methacryloyloxyethyl)-

2'-(trimethylammonium)ethyl phosphate inner salt-co-3-chloro-2-hydroxypropyl methacrylate (1:1)

2(methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate inner salt (7.46g, 25.3 mmole), 3-chloro-2-hydroxypropyl methacrylate (4.51g, 25.3 mmole) and p-toluene sulphonic acid monohydrate (0.1048g, 0.55 mmole) were dissolved in methanol (101ml). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes. 2,2'-azobis(2-methylpropionitrile) (0.0843g, 0.51mmole) was added and the flow of nitrogen was reduced to 10ml/min, the reaction temperature was raised to 60°C. This temperature and nitrogen flow rate were maintained for 16 hours.

The polymer was isolated from this mixture by precipitation in acetone (1500ml), vacuum filtration and drying. The polymer was redissolved in methanol (40ml) and isolated as before using acetone (1000ml).

The resulting polymer, obtained in 62% yield was a white solid.

NMR(200MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 4.3-4.0 (b), 3.6-3.8 (b), 3.3 (s), 1.6-2.4(b), 1.0-1.5(b), 0.7-1.0(b).

IR(cm⁻¹, KBr disc) 3416, 2959, 1727, 1655, 1490, 1247, 1165, 1088, 968, 792, 748.

25

Example 10

Preparation of poly(2(methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate inner salt-co-7 dodecylmethacrylate (1:2)

WO 93/01221

PCT/GB92/01215

- 82 -

The polymer was prepared by a method analogous to that described in Examples 4 and 6 using 2(methacryloyloxyethyl)-
2' (trimethylammonium)ethyl phosphate inner salt (8.41g, 0.0285
mole) and n-dodecylmethacrylate (14.31g, 0.0572 mole) dissolved
5 in propan-2-ol (160ml) and ethyl acetate (60ml).

The resulting polymer, obtained in 35% yield was a white powder.

NMR(100MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4(b), 3.8-4.2(b), 3.6-
3.8(b), 3.3(s), 2.25(s), 1.8-2.2(b), 1.5-1.8(b), 1.2-1.5(s), 0.8-
10 1.0(s)

IR(cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246,
1156, 1089, 968, 788.

Elemental Analysis

	theory	C 65.1	H 9.0	N 1.8	P 3.9
15	actual	C 54.9	H 8.5	N 1.9	P 4.4

Relative Viscosity (chloroform/ethanol 50:50, 30°C) 1.18.

The polymer may be crosslinked by gamma-irradiation or exposure to UV light which renders the polymer insoluble in dichloromethane/methanol.

20 A sample of stainless steel treated with the polymer showed a reduction in protein adsorption of 68% (determined by the enzyme immunoassay described above) and a reduction in platelet activation of 100% (determined by the platelet activation assay described above, using anti GMP 140) compared
25 to untreated material. A sample of PVC coated with the polymer showed a reduction in protein adsorption of 60% compared to untreated material as determined by the same assay technique.

Example 11

Preparation of poly(2(acryloyloxyethyl)-

2'(trimethylammonium)ethyl phosphate inner salt -co- n-dodecyl methacrylate) (1:2)

5 The polymer was prepared by a method analogous to that described in Examples 4 and 6 using 2(acryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt (3.0g, 0.0107 mole) and n-dodecyl methacrylate (5.42g, 0.0214 mole) dissolved in propan-2-ol (53ml) and ethyl acetate (22ml).

10 The resulting polymer, obtained in 58% yield was a white solid.

NMR(100MHz,d,ppm,CD₃OD/CDCl₃) 4.2-4.4(b),3.8-4.2(b),3.6-3.8(b),3.3(s),1.8-2.2(b),1.5-1.8(b),1.2-1.5(s),0.8-1.0(s)

IR(CM⁻¹,KBr disc) 3430, 2929, 2854, 1732, 1469, 1246,
15 1156, 1089, 968, 788.

Elemental Analysis

theory	C 64.9	H 8.7	N 1.8	P 4.0
actual	C 57.8	H 9.8	N 2.1	P 4.9

A sample of stainless steel treated with the polymer
20 showed a reduction in protein adsorption of 53% (determined by the enzyme immunoassay described above) and a reduction in platelet activation of 100% (determined by the platelet activation assay described above, using anti-GMP140) compared to untreated material. A sample of PVC treated with the
25 polymer showed a reduction in protein adsorption of 68% and a reduction in platelet activation of 100% compared to untreated material determined by the same assay techniques.

Example 12

Preparation of poly(2(methacryloyloxyethyl)-

WO 93/01221

PCT/GB92/01215

- 84 -

2' (trimethylammonium)ethyl phosphate inner salt -co- n-hexyl methacrylate (1:2)

The polymer was prepared by a method analogous to that described in Examples 4 and 6 using 2(methacryloyloxyethyl)-
5 2' (trimethylammonium)ethyl phosphate inner salt (2.0g, 0.0068 mole) and n-hexyl methacrylate (2.31g, 0.0136 mole) dissolved in propan-2-ol (35.5ml) and ethyl acetate (14.5ml).

The resulting polymer, obtained in 34% yield was a white solid.

10 NMR (100MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-3.8 (b), 3.3 (s), 1.8-2.2 (b), 1.5-1.8 (b), 1.2-1.5 (s), 0.8-1.0 (s)

IR (cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246, 1156, 1089, 968, 788.

Elemental Analysis

15	theory	C 58.8	H 8.8	N 2.2	P 4.9
	actual	C 47.3	H 7.9	N 2.6	P 5.8

Example 13

Preparation of poly(2(methacryloyloxyethyl)-

20 2' (trimethylammonium)ethyl phosphate inner salt -co- n-octadecyl methacrylate) (1:2)

The polymer was prepared by a method analogous to that described in Example 5 using 2(methacryloyloxyethyl)-
2' (trimethylammonium)ethyl phosphate inner salt (3.0g, 0.0102
25 mole) and n-octadecyl methacrylate (6.90g, 0.0204 mole) dissolved in methanol (30ml) and THF (70ml). The reaction mixture rate was maintained for 40 hours at 60°C. The polymer was isolated from this mixture by precipitation in acetone (1200ml), vacuum filtration and drying. The resulting polymer,

WO 93/01221

PCT/GB92/01215

- 85 -

obtained in 55% yield was a white solid.

NMR(100MHz,d,ppm,CD₃OD/CDCl₃) 4.2-4.4(b), 3.8-4.2(b), 3.6-
3.8(b), 3.3(s), 1.8-2.2(b), 1.5-1.8(b), 1.2-1.5(s), 0.8-1.0(s)

IR(cm⁻¹,KBr disc) 3430, 2929, 2854, 1732, 1469, 1246,
5 1156, 1089, 968, 788.

Example 14

Preparation of poly(2(methacryloyloxyethyl)-

2'(trimethylammonium)ethyl phosphate inner salt -co- n-dodecyl
10 methacrylate -co- 2 hydroxyethylmethacrylate) (17:75:8)

The polymer was prepared by a method analogous to
Examples 4 and 6, using 2(methacryloyloxyethyl)-
2'(trimethylammonium)ethyl phosphate inner salt (2.0g, 0.0068
mole), n-dodecyl methacrylate (7.65g, 0.0301 mole) and 2-
15 hydroxyethyl methacrylate (0.42g, 0.0032 mole) dissolved in
propan-2-ol(70ml) and ethyl acetate (30ml).

The resulting polymer, obtained in 53% yield was a white
solid.

NMR(100MHz,d,ppm,CD₃OD/CDCl₃) 4.2-4.4(b), 3.8-4.2(b), 3.6-
20 3.8(b), 3.3(s), 1.8-2.2(b), 1.5-1.8(b), 1.2-1.5(s), 0.8-1.0(s).

IR(cm⁻¹,KBr disc) 3435, 2925, 2860, 1729, 1468, 1243,
1152, 1089, 969, 791.

A coating solution of poly(2(methacryloyloxyethyl)-
2'(trimethylammonium)ethyl phosphate inner salt -co- n-dodecyl
25 methacrylate -co- 2-hydroxyethylmetacrylate) (0.5097g) in
propan-2-ol (50ml) was prepared. Aluminium sheet was washed
with propan-2-ol, hexane and water and dried, the coating
solution (0.5ml) was applied to pieces of the aluminium sheet
(7.5cm²) by a spin coating technique using a spin speed of

WO 93/01221

PCT/GB92/01215

- 86 -

1200rpm.

~ Example 15

Preparation of poly(2(methacryloyloxyethyl)-

5 2'(trimethylammonium)ethyl phosphate inner salt -co-
methacrylic acid (7:3)

The polymer was prepared by a method analogous to that of Examples 4 and 6 using 2(methacryloyloxyethyl)-
2'(trimethylammonium)ethyl phosphate inner salt (4.44g, 0.0149
10 mole), and methacrylic acid (0.54g, 0.0063 mole) dissolved in propan-2-ol (25ml) and water (25ml). The polymer was isolated by precipitation in acetone (500ml), redissolved in methanol (50ml) and isolated by precipitation in diethylether (500ml).

The resulting polymer, obtained in 30% yield was a white
15 solid.

NMR(100MHz, d, ppm, CD₃OD/CDCl₃) 4.2-4.4 (b), 3.8-4.2 (b), 3.6-
3.8 (b), 3.3 (s), 1.8-2.2 (b), 1.5-1.8 (b), 1.2-1.5 (s), 0.8-1.0 (s)

IR(cm⁻¹, KBr disc) 3430, 2929, 2854, 1732, 1469, 1246,
1156, 1089, 968, 788

20 This polymer was used to treat cellulose film which had been treated with 2-aminoethyl methacrylate as follows:

A section of cellulose dialysis membrane (4 x 6cm) was taken, and placed into a solution of 2-aminopropylmethacrylate (3.34g) and ceric ammonium nitrate (0.05g) in distilled water
25 (20ml). The solution was deoxygenated with N₂ for 10 minutes, then the vessel was sealed, and left at room temperature for 2 hours. The cellulose sample was then removed from the solution, then washed extensively in distilled water for 24 hours.

The presence of amine hydrochloride moieties on the grafted sample was demonstrated by the differential uptake of anionic and cationic dyes (Trypton blue and methylene blue respectively).

5 Strips of the functionalised cellulose (0.5cm x 2cm) were placed in a 10% w/w solution of the polymer in water. The samples were left to stand at room temperature for 1 hour, then washed extensively in distilled water (200ml) for 2 hours.

Following the aqueous wash, the treated cellulose was placed into a solution of acid molybdate spray reagent and left to stand for 1 hour, then removed and washed with distilled water. The presence of phosphate groups on the sample was demonstrated by the development of a blue colour.

15 Example 16

Preparation of poly(2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt -co- (2-methacryoyloxyethyl trimethylammonium chloride (7:3)

2(Methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt (4.45g, 0.0151 mole), 2-methacryoyloxyethyl trimethylammonium chloride (1.96g of a 75% aqueous solution, 0.0071 mole) were dissolved in ethanol (50ml). The solution was stirred (250rpm) at 23°C under a stream of nitrogen (50ml/min) for 30 minutes. 2,2'-Azo-bis(2-methylpropionitrile) (0.02g, 0.122 mole) was added and the flow of nitrogen was reduced to 10ml/min, the reaction temperature was raised to 60°C. This temperature and nitrogen flow rate were maintained for 40 hours.

The mixture was allowed to cool and filtered under

WO 93/01221

PCT/GB92/01215

- 88 -

vacuum. The polymer was isolated from this mixture by precipitation in diethylether (500ml), vacuum filtration and drying.

The resulting polymer, obtained in 68% yield was a white solid.

NMR(100MHz,d,ppm,CD₃OD/CDCl₃) 4.2-4.4(b), 3.8-4.2(b), 3.6-3.8(b), 3.3(s), 1.8-2.2(b), 1.5-1.8(b), 1.2-1.5(s), 0.8-1.0(s)

IR(cm⁻¹,KBr disc) 3430, 2929, 2854, 1732, 1469, 1246, 1156, 1089, 968, 788.

The polymer was used to treat cellulose film which had been treated with 3-sulfopropyl methacrylate potassium salt using the method described in Example 15 but using 3-sulfopropyl methacrylate potassium salt (4.92g) rather than 2-aminopropyl methacrylate.

The presence of sulphate moieties on the grafted sample was demonstrated by the differential uptake of anionic and cationic dyes (Trypton blue and methylene blue respectively).

Strips of the functionalised cellulose (0.5cm x 2cm) were treated with a 10% w/w solution of the polymer in water in an analogous manner to that described in Example 15 and the presence of phosphate groups was demonstrated in the same way.

Example 17

Preparation of poly(2(methacryloyloxyethyl)-

2'(trimethylammonium)ethyl phosphate inner salt -co- n-dodecyl methacrylate (14:86)

The polymer was prepared by a method analogous to that

WO 93/01221

PCT/GB92/01215

- 89 -

described in Example 1 using a comonomer mixture consisting of 2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt and n-dodecyl methacrylate in a molar ratio of 14:86 using propan-2-ol/ethylacetate solvent.

5 A PVC substrate was coated with the polymer using a method analogous to that described in Example 3.

Comparative Example

The fibrinogen adsorption and C-reactive protein binding to PVC substrates coated with polymers of the invention in accordance with Examples 3 and 17 was compared with that for 10 copolymers of 2(methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate, inner salt and butyl methacrylate in a molar ratio of 1:2 (Comparison A) and 14:86 (Comparison B). The comparison copolymers were prepared by a 15 method analogous to that described in Example 1 and coated onto the PVC substrate using a method analogous to that described in Example 3. All the copolymers were prepared using the procedure described under Example 1 using a propan-2-ol/ethylacetate solvent. The results are shown in the 20 following Table:

	Example		Comparison	
	3	17	A	B
25 Fibrinogen Adsorption	87	60	82	72
C-reactive Protein	0.094	0.043	0.101	0.139
Protein Index ($\times 10^3$)	1.1	0.7	1.9	1.9

The results were obtained using the assay techniques

WO 93/01221

PCT/GB92/01215

- 90 -

described above after incubation in PBS for 24 hours. The
fibrinogen adsorption results are expressed as a percentage
reduction in optical density relative to untreated
Polyvinylidene chloride. C-reactive protein results are
5 expressed as absorbance due to C-reactive protein; a positive
control showed CRP binding. Protein index is the ratio of C-
reactive protein binding to fibrinogen adsorption.

These results show that in order to obtain a good
reduction in protein adsorption using copolymers containing
10 butylmethacrylate a high C-reactive protein binding must be
accepted. In contrast, using longer chain alkyl monomers good
reduction in protein adsorption and low C-reactive protein
binding are obtained as well as good adhesion to the substrate
and low swelling in aqueous environments.

15 Copolymers containing comonomers comprising short chain
alkyl groups of up to 4 carbon atoms as potential physisorbable
groups such as butyl methacrylate, in smaller molar
proportions, exhibit poor adhesion to hydrophobic substrates
and are subject to high swelling in aqueous environments which
20 renders them unsuitable for use in coating surfaces.

Example 18

Polymers from examples 1 and 4 were coated onto PVC
tubing and their performance assessed in an extracorporeal
system using a left heart bypass procedure in a calf model.
25 Blood was pumped around the system continuously at
physiological temperature in the absence of anticoagulant at a
rate of 3.5 litre per minute. Parameters associated with the
condition of circulating blood were measured throughout the
experiments.

WO 93/01221

PCT/GB92/01215

- 91 -

Virtually all the parameters tested gave results which showed that the presence of the coated circuit had little or no effect on the blood and the physiological function of the animals (typically three identical experiments were run on consecutive days and were very reproducible from animal to animal).

In comparison with the above an uncoated circuit cannot be successfully run for a continuous 6 hour period without the use of anticoagulant. Also blood parameters are very adversely affected in a short period of time.

Results of protein adsorption tests and macroscopic observation of circuit components indicate that both coatings perform at least as well as heparinised tubing and that, in areas of the circuit where high turbulence in the flow of the blood occurs, fewer clots were found.

Reference Example 1

Preparation of 2(methacryloyloxyethyl)-2'(trimethylammonium ethyl phosphate inner salt

The preparation is illustrated by the reaction scheme A which follows.

a) 2-Chloro-1,3-dioxaphospholane (1)

In a flask fitted with a pressure equalising dropping funnel, reflux condenser (fitted with a CaCl_2 guard tube) and magnetic stirrer, was placed a solution of phosphorus trichloride (220ml; 346.3g; 2.52mol) in dichloromethane (500ml). Ethylene glycol (139ml; 154.7g, 2.49mol) was then added dropwise via the dropping funnel at such a rate that the evolution of HCl did

WO 93/01221

PCT/GB92/01215

- 92 -

not become too excessive. On the addition of the ethylene glycol, the condenser was arranged for distillation, and the ~dichloromethane removed at atmospheric pressure. When the distillate temperature reached 60°C the flask was arranged for
5 vacuum distillation using a water pump, Distillation then gave 2-chloro-1,3-dioxaphospholane (158ml; 224.5g; 71.3%) as a colourless mobile liquid (which fumes in moist air) b.pt. 36-40°C/21mm Hg. [cf 45.5-47°C/20mm Hg, Lucas et al, J. Am. Chem. Soc., 72, 5491, (1950)].

10 IR (cm⁻¹, thin film) 2980, 2905, 1470, 1210, 1005, 930, 813, 770.

b) 2-Chloro-2-oxo-1,3,2-dioxaphospholane (2)

In a flask fitted with a magnetic stirrer, reflux condenser (fitted with a CaCl₂ guard tube) and sintered glass
15 gas inlet tube, was placed a solution of 2-chloro-1,3-2-dioxaphospholane (100.8g; 0.797mol) in dry benzene (200ml). The solution was stirred and a steady stream of oxygen was bubbled through the solution. The reaction was mildly exothermic, and temperature control was achieved by allowing
20 the solvent to reflux. The oxygen was passed through the reaction mixture for 6 hours. The solvent was removed by rotary evaporation, and the colourless mobile residue distilled to give 2-chloro-2-oxo-1,3,2-dioxaphospholane (2) (87.41g; 77%) as a colourless mobile liquid -b.pt 95-97°C/0.2mbar [c.f.
25 102.5-105°C/1mbar (Edmundson, Chem. Ind. (London)), 1828 (1962); 79°C/0.4mbar (Umeda et al., Makromol. Chem. Rapid Commun., 3, 457, (1982)].

IR(cm⁻¹, thin film) 2990, 2910, 1475, 1370, 1310, 1220, 1030, 930, 865, 830.

WO 93/01221

PCT/GB92/01215

- 93 -

c) 2(2-Oxo-1,3,2-dioxaphospholan-2-yloxy)ethyl methacrylate
(3)

In a flask fitted with a magnetic stirrer, low temperature thermometer, and a pressure equalising funnel fitted with a silica gel guard tube, was placed a solution of 2-hydroxyethylmethacrylate (20.00g, 0.154mol) and triethylamine (15.60g; 0.154mol) in dry diethyl ether (300ml). The solution was stirred and cooled to between -20°C and -30°C. A solution of freshly distilled 2-chloro-2-oxo-1,3,2-dioxaphospholane(2) (21.9g; 0.154 mol) in dry diethyl ether (20ml) was then added dropwise over 30 minutes, the temperature being held at -20°C during the addition. Stirring was continued at this temperature for a further 1 hour and then for a further hour as the reaction mixture was allowed to warm to room temperature.

The precipitated triethylamine hydrochloride was removed by filtration, and was washed well with dry ether. The ether was removed from the combined filtrate and washings by rotary evaporation. The cloudy oil residue was then shaken for 5 minutes with dry diethyl ether (50ml) to precipitate a further crop of triethylamine hydrochloride, which was again removed by filtration. Removal of the ether on the rotary evaporator gave (3) (34.18g; 94.3%) as a colourless viscous oil.

IR (cm⁻¹, thin film) 1720, 1640, 1450, 1360, 1310, 1290, 1170, 1030, 930, 850.

¹H NMR (CDCl₃; 60MHz, δ ppm) 1.95 (s, 3H), 4.25-4.70 (m, 8H), 5.70 (m, 1H), 6.25 (m, 1H).

Rf 0.9 (SiO₂, eluting with 10% methanol:90% dichloromethane; spot visualised with molybdenum blue spray reagent and with iodine vapour).

WO 93/01221

PCT/GB92/01215

- 94 -

d) 2(Methacryloyloxyethyl)-2'(trimethylammonium)ethyl phosphate inner salt (4).

The phospholane (3) (67.20g; 0.285 mol) was dissolved in 100 ml of dry acetonitrile, and placed in a heavy walled tissue culture bottle. The phospholane solution was then treated with a solution of anhydrous trimethylamine (25.74g; 0.436 mol) in dry acetonitrile (100ml). The vessel was then sealed, and placed in a water bath held at 50°C for 30 hours. The vessel was opened, and the solution brought to the boil. The solution was filtered whilst hot, and then set aside for crystallisation.

The product was collected by filtration, and most of the solvent removed by suction. The wet product was then washed thoroughly with anhydrous ether, then dried under reduced pressure, to give (4) as a white amorphous, hygroscopic solid (51.16g; 61%). Evaporation of the mother liquor gave a very viscous oil (20.00g; 23%), from which further product (4) crystallised on standing at -20°C. TLC (silica gel plates, eluting with methanol/dichloromethane (1:1 v/v)) showed one spot R_f 0.1, which was revealed with Dragendorff's reagent, Molybdenum blue spray reagent, and iodine vapour.

IR(cm⁻¹ 1720, 1640, 1320, 1300, 1230, 1170, 970, 750.

NMR (D₂O; 60MHz; δ ppm) 2.0 (s,3H), 3.27 (s,9H) 3.60-4.50 (m, 8H), 5.80, (m,1H) and 6.25 (m,1H).

CHN Found: C 42.98%, H 7.88%, N 4.42%, P 10.51%.

CHN Theory: C 44.75%, H 7.46%, N 4.75%, P 10.51%.

Reference Example 2:

Synthesis of dimethyl(2-methacroyloxyethyl)-(1(2-sulphopropyl))

WO 93/01221

PCT/GB92/01215

- 95 -

ammonium betaine inner salt

2(Dimethylamino)ethylmethacrylate was vacuum distilled and then dissolved in 0.1M dichloromethane. To this solution was added an equimolar amount of propane sultone. The betaine slowly precipitated out of solution and was recovered by filtration and washed with cold dichloromethane. The reaction is shown in Reaction Scheme B.

Reference Example 3

Preparation of 1[4(4'-vinylbenzyloxy)butane]-2"-

10 (trimethylammonium)ethyl phosphate inner salt.

The synthesis is depicted in Reaction Scheme C.

4-Hydroxy-1(4'-vinylbenzyloxy)butane (5)

Butanediol (40ml; 40.68g; 0.452mol) was stirred in a 100ml round bottomed flask, and treated portionwise with potassium butoxide (17.60g; 0.144mol). The initial reaction was exothermic. The reaction mixture was stirred for 1.5 hours at room temperature. The resulting cloudy solution was then treated with chloromethyl styrene (20.00g; 0.131 mol). The styrene formed an upper, pale green layer, (the colouration being due to the presence of inhibitor), whose color darkened considerably on the addition of 18-crown-6 (0.49g; 1.86×10^{-3} mole). The flask was stoppered, protected from light, and stirred for 28 hours at room temperature. The mixture was then poured into water (120 ml) and extracted with dichloromethane (4x50ml). The combined organic extracts were dried (MgSO_4) and evaporated to give viscous yellow oil (932.7g). This oil was distilled from a small amount of CuCl to give a product showing some impurities on TLC. The oil was then chromatographed on silica gel, initially eluting with dichloromethane/petrol (1:1)

WO 93/01221

PCT/GB92/01215

- 96 -

to remove the impurities. The product was then eluted off the column with ethyl acetate/petrol (1:1). Evaporation of the solvent gave a colourless oil, which was distilled to give the desired styrylbutyl alcohol as a colourless oil b.pt. 150-
5 152°/0.4mbar. Yield 18.70g; 69.2%.

NMR (60MHz: CDCl₃) 1.55 (m, 4H C-CH₂-C); 3.50 (m, 5H, 1H exch.; O-CH₂-, O-H), 4.45 (s, 2H; Ar-CH₂-), 5.50 (dd, 2H, vinylic), 6.75 (dd, vinylic), 7.40 (m, 4H, Ar-H).

10

IR 3402, 2938, 2888, 1631, 1602, 1582, 1511, 1480, 1445, 1382, 1320, 1116, 1063, 920, 907, 827, 801, 716 and 667 cm⁻¹

4(2-Oxo-1,2,3-dioxaphospholane-2-yloxy)-1(4'-vinylbenzyloxy)butane (6)

15 4-Hydroxy-1(4'-vinylbenzyloxy)butane (5) (10.03g; 48.69 mmol) and dried triethylamine (4.92g, 48.69 mmol) were dissolved in dry diethyl ether (150ml) and the resulting solution placed in a rigorously dried flask. The solution was cooled to -30°C and 2-chloro-2-oxo-1,3,2-dioxaphospholane (6.94g; 48.69 mmol) added
20 dropwise over 30 minutes, the temperature being held at -30°C. The reaction mixture was then stirred for a further 2 hours, during which time the temperature was allowed to rise to 10°C. The mixture was filtered and the precipitate washed with dry ether. The filtrate was evaporated (20°C / 21mm) to give a
25 cloudy oil. The residue was shaken with 50ml of dry ether and refiltered. Evaporation of the filtrate gave the product as a viscous yellow oil (13.73 g; 90.4%).

TLC (eluting with 10% methanol 90% dichloromethane) showed one major spot, which stained with acid molybdate

WO 93/01221

PCT/GB92/01215

- 97 -

reagent (Rf 0.61), IR (thin film) 3458, 2945, 2917, 2860, 1630, 1602, 1581, 1475, 1419, 1363, 1283, 1103, 1032, 820, 842, 807, 800, 715, 610 and 421 cm⁻¹.

1[4(4'-Vinylbenzyloxy)butane]-2"(trimethylammonium)ethyl

5 phosphate inner salt (7)

Trimethylamine (2.00g, 33.9 mmol) was distilled into a reaction vessel, and frozen with liquid nitrogen. A solution of the 4(2-oxo-1,3,2-dioxaphospholane-2-yloxy)-1-(4'-vinylbenzyloxy)butane (6) (10.00g, 32.1 mmol) in anhydrous
10 acetonitrile (40ml) was then added to the reaction vessel, which was then sealed and placed in a thermostatted water bath (50°C for 50 hours). The reaction vessel was then cooled to room temperature, opened, and the reaction mixture evaporated to about half its original volume (21 mm pressure). The
15 concentrated solution was then stirred at room temperature, whilst anhydrous ether (200ml) was added dropwise to precipitate the product as a viscous oil. The mixture was then left for several hours at -10°C. The product was collected by decanting off the supernatant solid. TLC (eluting with
20 methanol/dichloromethane 1:1) showed one major spot at Rf 0.0-0.1 which stained with both Dragendorffs reagent and acid molybdate.

25 Reference Example 4

Preparation of 2(acryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate inner salt

The synthesis is essentially analogous to that described
30 in Reference Example 1 and uses a synthetic strategy analogous

WO 93/01221

PCT/GB92/01215

- 98 -

to that shown in Reaction Scheme A.

(a) 2-(2-Oxo-1,3,2-dioxaphospholan-2-yloxy)ethyl acrylate

2-Hydroxyethyl acrylate (11.5ml, 0.1M) and triethylamine (14.6 ml) in dry diethyl ether (250ml) were cooled to -25°C under nitrogen as a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (14.3g) in dry diethyl ether was added over 20 minutes. The mixture was stirred for a further 1 hour at -20°C and then allowed to warm to 10°C over a further hour. The precipitate was filtered, washed with ethyl acetate (100ml) and the combined filtrate and washings evaporated under reduced pressure to give a pale yellow oil (21g).

¹H NMR (200MHz) d (CD₃CN) 6.4 (1H,dd), 6.2 (1H,dd), 5.9 (1H,dd), 4.0-3.6 (8H,complex) ppm.

(b) 2-(Acryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate, inner salt.

2-(2-Oxo-1,3,2-dioxaphospholan-2-yloxy) ethyl acrylate (21g, 0.095M) in acetonitrile (50ml) was treated with a solution of triethylamine (12.1g) in acetonitrile (150ml) in a pressure reactor at 50°C for 17 hours. The mixture was cooled and some of the excess triethylamine removed by evaporation under reduced pressure.

The solid material was filtered under nitrogen, washed with acetonitrile (20ml) and diethylether (50ml) and then dried under reduced pressure to give a colourless oil (12.1g, 45%).

¹H NMR (200MHz) d (D₂O) 6.45 (1H,dd, J1.2 and 17.1 Hz), 6.25 (1H,dd, J1.2 and 10.25Hz), 6.02 (1H,dd, J1.23 and 10.25Hz), 4.4 (2H,m), 4.3 (2H, m), 4.2 (2H,m) 3.6(2H,m) and 3.2(9H,s) ppm.

Reference Example 5

Dodec-7-yn-1-ol Methacrylate

To dodec-7-yn-1-ol (25g) in dichloromethane (60ml) was added distilled triethylamine (14.1g). The mixture was cooled in an ice bath (0.5°C) and stirred as distilled methacryloyl chloride (16.2g) in dichloromethane (50ml) was added over 10 minutes. The temperature of the reaction was allowed to warm to ambient and the mixture stirred for two hours. Water (150ml) was added and the organic layer was removed and successively extracted with water (2 x 150ml) and saturated sodium bicarbonate solution (2 x 150ml), washed with brine (150ml) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to give a pale yellow oily liquid which was distilled under reduced pressure (0.18 mBar, 106-110°C) in the presence of copper (1) chloride to give dodec-7-yn-1-ol methacrylate, 17g, 50% yield.

¹H-NMR (200MHz, d, ppm, CDCl₃): 0.90 (t, 3H), 1.45 (m, 10H), 1.70 (m, 2H), 1.95 (s, 3H), 2.15 (m, 6H), 4.15 (t, 2H), 5.55 (s, 1H), 6.10 (s, 1H).

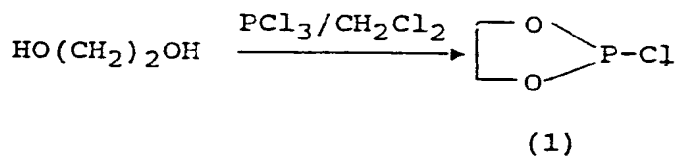
WO 93/01221

PCT/GB92/01215

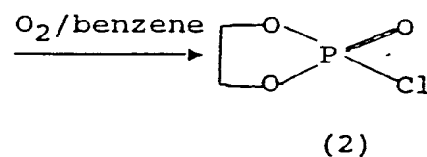
- 100 -

Reaction Scheme A

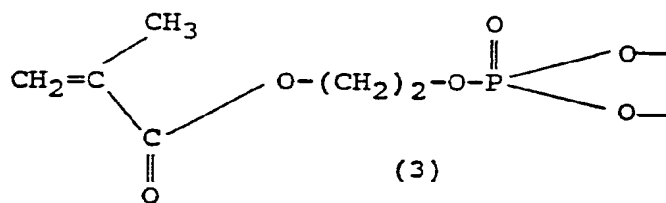
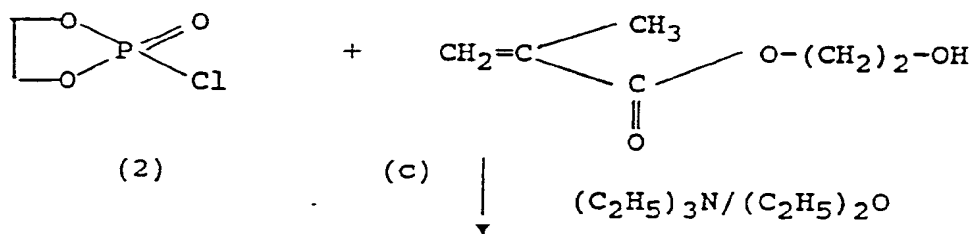
Step (a)



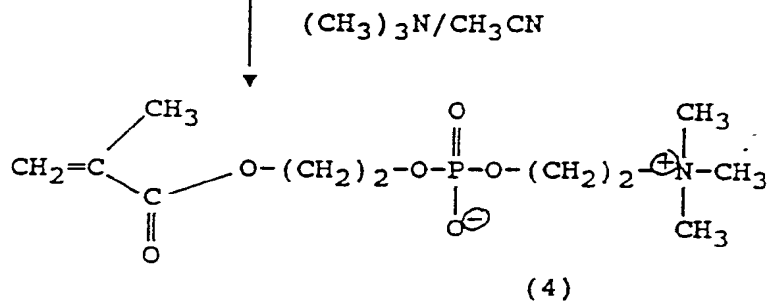
Step (b)



Step (c)



Step (d)



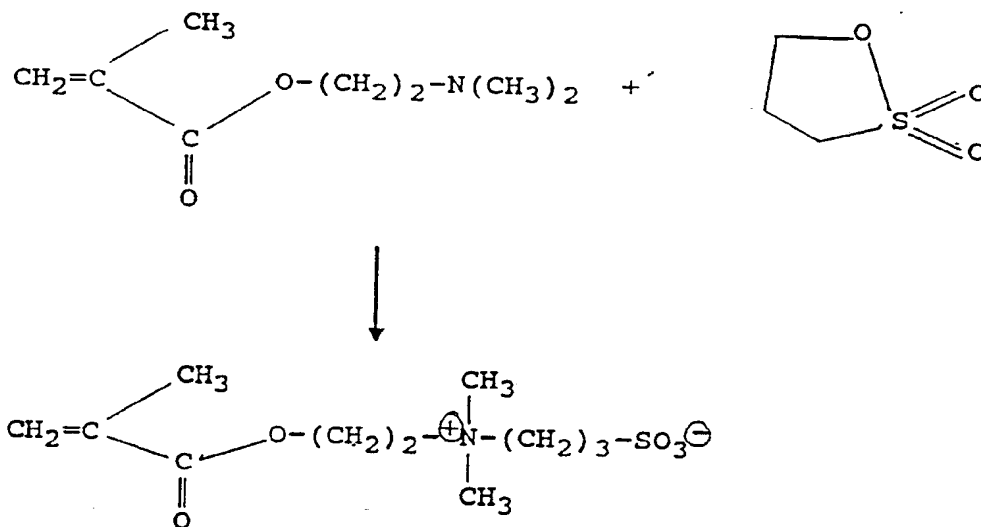
Steps (a) to (d) correspond with the steps in Reference Example 1

WO 93/01221

PCT/GB92/01215

- 101 -

Reaction Scheme B



5

HO(CH₂)₄OH

(5)

10

15

20

Me₃N

25

(6)

(7)

WO 93/01221

PCT/GB92/01215

- 103 -

CLAIMS

1. A polymer of one or more radical polymerisable monomers which polymer has pendant groups bearing a centre of permanent positive charge and other pendant groups
5 capable of stably binding the polymer to a surface.

2. A polymer according to claim 1 which comprises pendant groups in which the centre of permanent positive charge is in a zwitterionic group.

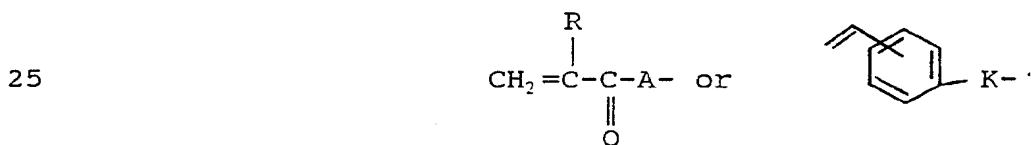
3. A polymer according to claim 2 in which
10 comprises residues of a comonomer bearing a zwitterionic group of the formula:



wherein B is a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene chain optionally containing one or
15 more fluorine atoms up to and including perfluorinated chains or, if X contains a carbon-carbon chain between B and the centre of permanent positive charge or if Y contains a terminal carbon atom bonded to B, a valence bond;

20 X is a zwitterionic group and

Y is an ethylenically unsaturated polymerisable group selected from



wherein:

R is hydrogen or a C₁-C₄ alkyl group;

30 A is -O- or -NR¹- where R¹ is hydrogen or a C₁-C₄

WO 93/01221

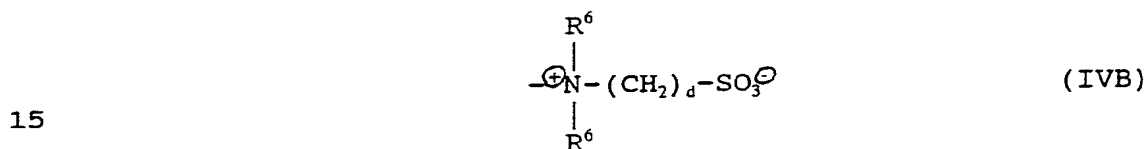
PCT/GB92/01215

- 104 -

alkyl group or R^1 is $-B-X$ where B and X are as defined above; and

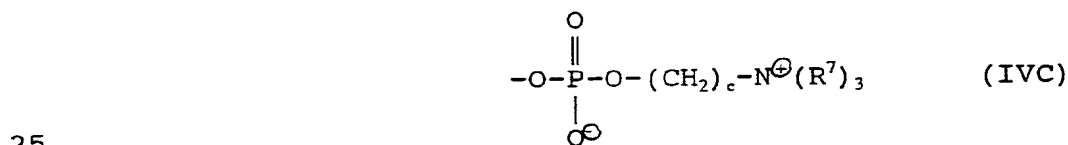
K is a group $-(CH_2)_pOC(O)-$, $-(CH_2)_pC(O)O-$,
 $-(CH_2)_pOC(O)O-$, $-(CH_2)_pNR^2-$, $-(CH_2)_pNR^2C(O)-$,
 5 $-(CH_2)_pC(O)NR^2-$, $-(CH_2)_pNR^2C(O)O-$, $-(CH_2)_pOC(O)NR^2-$,
 $-(CH_2)_pNR^2C(O)NR^2-$, (in which the groups R^2 are the same or different) $-(CH_2)_pO-$, $-(CH_2)_pSO_3-$, or, optionally in combination with B, a valence bond and p is from 1 to 12 and R^2 is hydrogen or a C_1-C_4 alkyl group.

10 4. A polymer to claim 3 in which in the comonomer of formula (I), X is a group of formula (IVB)



where the groups R^6 are the same or different and each is hydrogen or C_{1-4} alkyl and d is from 2 to 4;

20 a group of formula (IVC)



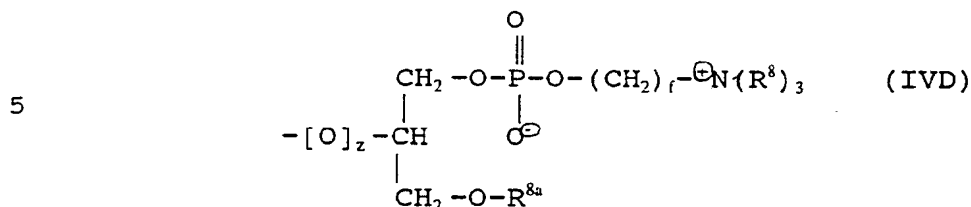
where the groups R^7 are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4;

WO 93/01221

PCT/GB92/01215

- 105 -

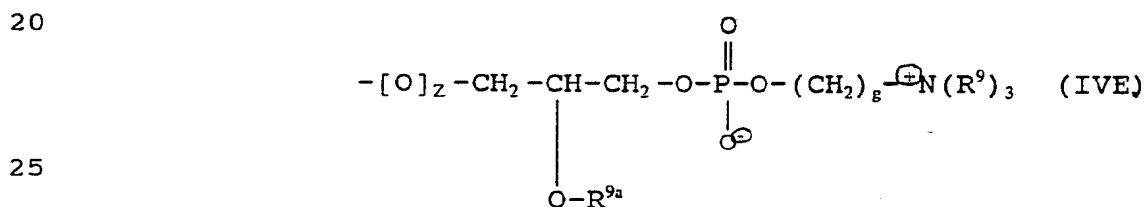
a group of formula (IVD):



wherein the groups R^8 are the same or different and each is hydrogen or C_{1-4} alkyl, R^{8a} is hydrogen or a group $-\text{C}(\text{O})\text{B}^1\text{R}^{8b}$ where R^{8b} is hydrogen or methyl, B^1 is a valence bond or straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and f is from 1 to 4; and

if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0, if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1;

a group of formula (IVE):



wherein the groups R^9 are the same or different and each is hydrogen or $\text{C}_1\text{-C}_4$ alkyl, R^{9a} is hydrogen or a group $-\text{C}(\text{O})\text{B}^2\text{R}^{9b}$, where R^{9b} is hydrogen or methyl, B^2 is a valence bond or a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and g is from 1 to 4; and

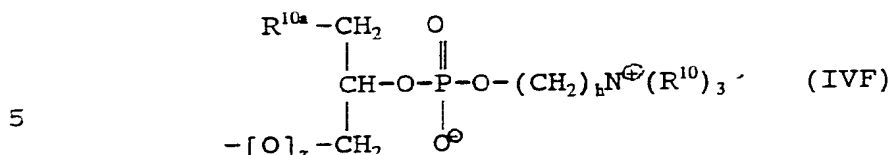
if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0 if X is directly bonded to an oxygen or nitrogen atom and otherwise Z is 1; or

WO 93/01221

PCT/GB92/01215

- 106 -

a group of formula (IVF)



wherein the groups R^{10} are the same or different and each is hydrogen or C_{1-4} alkyl, R^{10a} is hydrogen or a group $-\text{C}(\text{O})\text{B}^3\text{R}^{10b}$ where R^{10b} is hydrogen or methyl, B^3 is a valence bond or a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group, and h is from 1 to 4; and

if B is other than a valence bond Z is 1 and if B is a valence bond Z is 0 if X is directly bonded to the oxygen or nitrogen and otherwise Z is 1.

5. A polymer according to claim 4 comprising residues of a comonomer of formula (IVC).

6. A polymer according to any one of the preceding claims, obtainable by

(i) copolymerising a radical polymerisable comonomer containing a zwitterionic group and a radical polymerisable comonomer containing a radical polymerisable moiety and an alkyl group of 6 or more carbon atoms, which alkyl group optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms and optionally one or more carbon-carbon double or triple bonds, or a siloxane group; or

WO 93/01221

PCT/GB92/01215

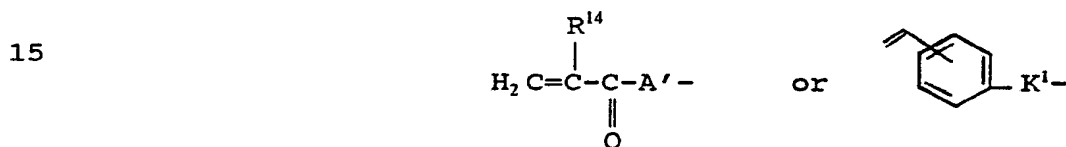
- 107 -

(ii) polymerising a radical polymerisable monomer containing a zwitterionic group and an alkyl group of 6 or more carbon atoms, which alkyl group optionally contains one or more etheric oxygen atoms, or a fluoroalkyl group which optionally contains one or more etheric oxygen atoms, or a siloxane group.

7. A polymer according to claim 6 which is obtainable by copolymerising a comonomer containing a zwitterionic group and a comonomer containing an alkyl, fluoroalkyl or siloxane group of general formula (VI)



where Y^1 is an ethylenically unsaturated polymerisable group selected from



where R^{14} is hydrogen or C_1 - C_4 alkyl,

A' is $-O-$ or $-NR^{15}-$ where R^{15} is hydrogen or a C_1 - C_4 alkyl group or R^{15} is a group Q ;

K^1 is a group $-(CH_2)_lOC(O)-$, $-(CH_2)_lC(O)O-$, $-(CH_2)_lOC(O)O-$, $-(CH_2)_lNR^{16}-$, $-(CH_2)_lNR^{16}C(O)-$, $-(CH_2)_lC(O)NR^{16}-$, $-(CH_2)_lNR^{16}C(O)O-$, $-(CH_2)_lOC(O)NR^{16}-$, $-(CH_2)_lNR^{16}C(O)NR^{16}-$ (in which the groups R^{16} are the same or different), $-(CH_2)_lO-$, $-(CH_2)_lSO_3-$, a valence bond and l is from 1 to 12 and R^{16} is hydrogen or a C_1 - C_4 alkyl group; and Q is

(a) straight or branched alkyl, alkoxyalkyl or

WO 93/01221

PCT/GB92/01215

- 108 -

(oligo-alkoxy)alkyl chain containing 6 or more carbon atoms unsubstituted or substituted by one or more fluorine atoms and optionally containing one or more carbon-carbon double or triple bonds; or

5 (b) a siloxane group $-(CR^{16a})_{qq}(SiR^{16b})_p(OSiR^{16b})_{pp}R^{16b}$ in which each group R^{16a} is the same or different and is hydrogen or alkyl of 1 to 4 carbon atoms, each group R^{16b} is alkyl of 1 to 4 carbon atoms, qq is from 1 to 6 and pp is from 0 to 49.

10 8. A polymer according to claim 7, in which, in the comonomer of formula (VI), Q is a straight or branched alkyl, alkoxyalkyl or (oligo-alkoxy)alkyl chain containing 6 or more carbon atoms, unsubstituted or substituted by one or more fluorine atoms.

15 9. A polymer according to any one of claims 1 to 5 which is obtainable by:

(i) copolymerising a radical polymerisable comonomer containing a zwitterionic group and a radical polymerisable comonomer bearing a reactive group capable of covalently binding the polymer to a surface; or

20

(ii) polymerising a radical polymerisable monomer containing a zwitterionic group and a reactive group capable of covalently binding the polymer to a surface.

25 10. A polymer according to claim 9 which is obtainable by copolymerising a comonomer containing a zwitterionic group and a comonomer containing a reactive group capable of covalently binding to a surface of general

WO 93/01221

PCT/GB92/01215

- 109 -

formula (IX)



where Y^2 is an ethylenically unsaturated polymerisable group selected from

5



10

where R^{19} is hydrogen or C_1 - C_4 alkyl,

K^2 is a group $-(CH_2)_qOC(O)-$, $-(CH)_qC(O)O-$,

$-(CH_2)_qOC(O)O-$, $-(CH_2)_qNR^{20}-$, $-(CH_2)_qNR^{20}C(O)-$,

$-(CH_2)_qC(O)NR^{20}-$, $-(CH_2)_qNR^{20}C(O)O-$, $-(CH_2)_qOC(O)NR^{20}-$,

15 $-(CH_2)_qNR^{20}C(O)NR^{20}-$ (in which the groups R^{20} are the same or different), $-(CH_2)_qO-$, or $-(CH_2)_qSO_3-$, or a valence bond and q is from 1 to 12 and R^{20} is hydrogen or a C_1 - C_4 alkyl group; and

20 Q^1 is a reactive group capable of reacting to provide covalent binding to a surface.

11. A polymer according to any one of claims 1 to 5 which is obtainable by:

25 i) copolymerising a radical polymerisable comonomer containing a zwitterionic group and a radical polymerisable comonomer bearing an ionic group capable of binding to a surface by ionic interaction; or

ii) polymerising a radical polymerisable monomer containing a zwitterionic group and an ionic group capable of binding to a surface by ionic interaction.

30

WO 93/01221

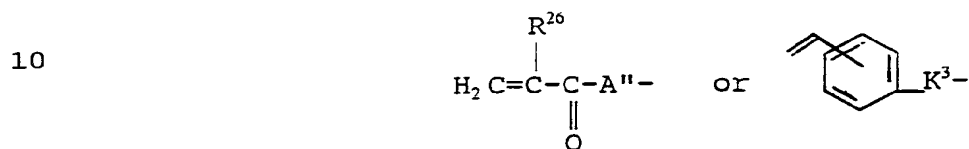
PCT/GB92/01215

- 110 -

12. A polymer according to claim 11 which is obtainable by copolymerising a comonomer containing a zwitterionic group and a comonomer bearing an ionic group capable of binding to a surface by ionic interaction of
5 general formula (XII)



where Y^2 is an ethylenically unsaturated polymerisable group selected from



15 where R^{26} is hydrogen or C_1-C_4 alkyl;

A'' is $-O-$ or $-NR^{27}-$, wherein R^{27} is hydrogen or a C_1-C_4 alkyl group or R^{27} is a group $-B^9-Q^5$;

B^9 is a valence bond a straight or branched alkylene, oxaalkylene or oligo-oxaalkylene group;

20 K^3 is a group $-(CH_2)_xOC(O)-$, $-(CH)_xC(O)O-$, $-(CH_2)_xOC(O)O-$, $-(CH_2)_xNR^{28}-$, $-(CH_2)_xNR^{28}C(O)-$, $-(CH_2)_xC(O)NR^{28}-$, $-(CH_2)_xNR^{28}C(O)O-$, $-(CH_2)_xOC(O)NR^{28}-$, $-(CH_2)_xNR^{28}C(O)NR^{28}-$ (in which the groups R^{28} are the same or different), $-(CH_2)_xO-$, $-(CH_2)_xSO_3-$, a valence bond, x is from
25 1 to 12 and R^{28} is hydrogen or a C_1-C_4 alkyl group; and

Q^5 is an ionic group capable of binding to a surface by ionic interaction.

13. A polymer according to any one of the preceding claims comprising

30

WO 93/01221

PCT/GB92/01215

- 111 -

- (a) residues of comonomer containing a zwitterionic group and of comonomer containing an alkyl, fluoroalkyl or siloxane group capable of binding the polymer to a surface in a molar ratio from 5:95 to 80:20;
- 5 (b) residues of comonomer containing a zwitterionic group and of comonomer containing a reactive group capable of binding the polymer to a surface by covalent bonding in a molar ratio from 5:95 to 95:5; or
- 10 (c) residues of comonomer containing a zwitterionic group and of comonomer containing an ionic group capable of binding the polymer to a surface by ionic interactions in a molar ratio from 5:95 to 95:5; and
- optionally 5% to 50% by mole of diluent comonomer residues and from 0.1% to 20% by mole of crosslinkable
- 15 comonomer residues provided that the total of diluent and crosslinkable comonomer residues does not exceed 50% by mole.
14. A polymer according to claim 9 or claim 10 bearing a bigand attached to the polymer by the residue of
- 20 a reactive group.
15. A polymer according to any one of the preceding claims when coated on a surface, bearing a ligand attached to the polymer by the residue of a reactive group.
16. A process for preparing a polymer which
- 25 comprises:
- (a) copolymerising a radical polymerisable comonomer containing a group bearing a centre of permanent

WO 93/01221

PCT/GB92/01215

- 112 -

... positive charge, a radical polymerisable comonomer containing a group capable of stably binding the polymer to a surface and optionally a diluent and/or crosslinkable comonomer; or

5 (b) polymerising a radical polymerisable monomer containing a group containing a group bearing a centre of permanent positive charge and a group capable of stably binding the polymer to a surface and optionally further comonomer containing a group capable of stably binding the
10 polymer to the surface and a diluent and/or a crosslinkable comonomer.

17. A process for biocompatibilising a surface which comprises:

(a) coating the surface with a polymer as claimed
15 in any one of claims 1 to 13 and where the polymer coating comprises additional unreacted reactive groups, optionally attaching a ligand to the surface by reaction with such reactive groups; or

(b) attaching a ligand to a polymer as claimed in
20 any one of claims 1 to 13 which comprises reactive groups by reaction with reactive groups, and coating the surface with the ligand-bearing polymer.

18. A finished device comprising a surface having coating thereon of a polymer as claimed in any one of
25 claims 1 to 15.

WO 93/01221

PCT/GB92/01215

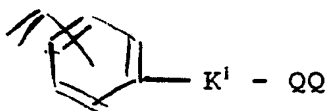
- 113 -

19. A compound of the formula:



or

5



(VIII A)

where R^{14} is hydrogen or $\text{C}_1\text{-C}_4$ alkyl,

A' is $-\text{O}-$ or $-\text{NR}^{15}-$ where R^{15} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group or R^{15} is a group Q;

K^1 is a group $-(\text{CH}_2)_1\text{OC}(\text{O})-$, $-(\text{CH}_2)_1\text{C}(\text{O})\text{O}-$,
 10 $-(\text{CH}_2)_1\text{OC}(\text{O})\text{O}-$, $-(\text{CH}_2)_1\text{NR}^{16}-$, $-(\text{CH}_2)_1\text{NR}^{16}\text{C}(\text{O})-$,
 $-(\text{CH}_2)_1\text{C}(\text{O})\text{NR}^{16}-$, $-(\text{CH}_2)_1\text{NR}^{16}\text{C}(\text{O})\text{O}-$, $-(\text{CH}_2)_1\text{OC}(\text{O})\text{NR}^{16}$,
 $-(\text{CH}_2)_1\text{NR}^{16}\text{C}(\text{O})\text{NR}^{16}-$ (in which the groups R^{16} are the same or
 different), $-(\text{CH}_2)_1\text{O}-$, $-(\text{CH}_2)_1\text{SO}_3-$, or a valence bond and 1
 is from 1 to 12 and R^{16} is hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group;
 15 and QQ is an alkynyl group containing 6 or more carbon
 atoms and one or two carbon-carbon triple bonds, provided
 that the acetylenic moieties are not directly bonded to A'
 or K^1 .

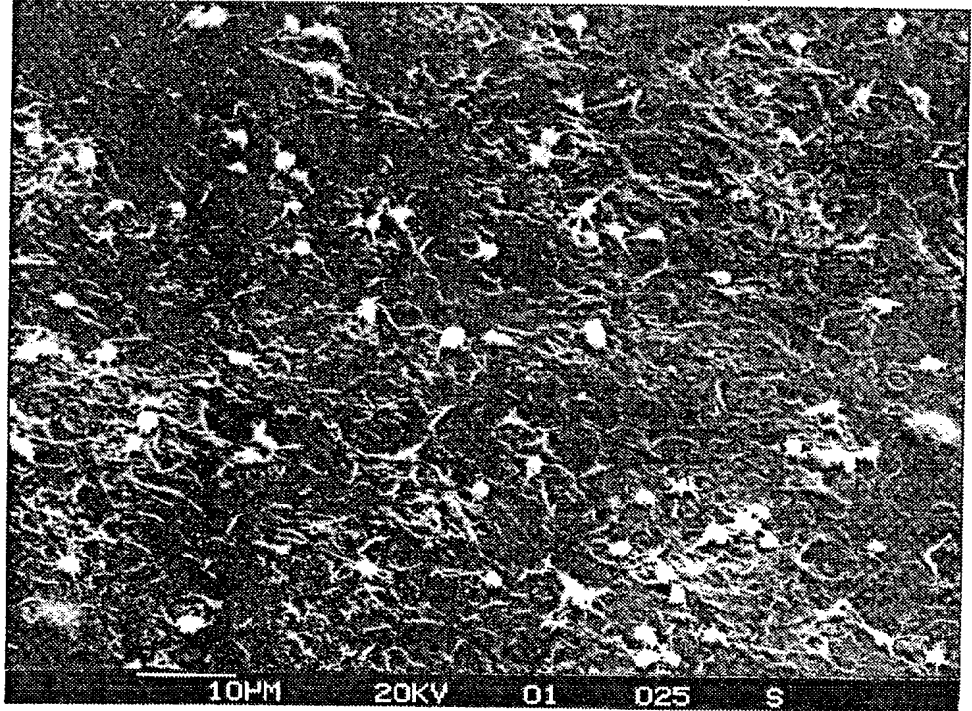
WO 93/01221

PCT/GB92/01215

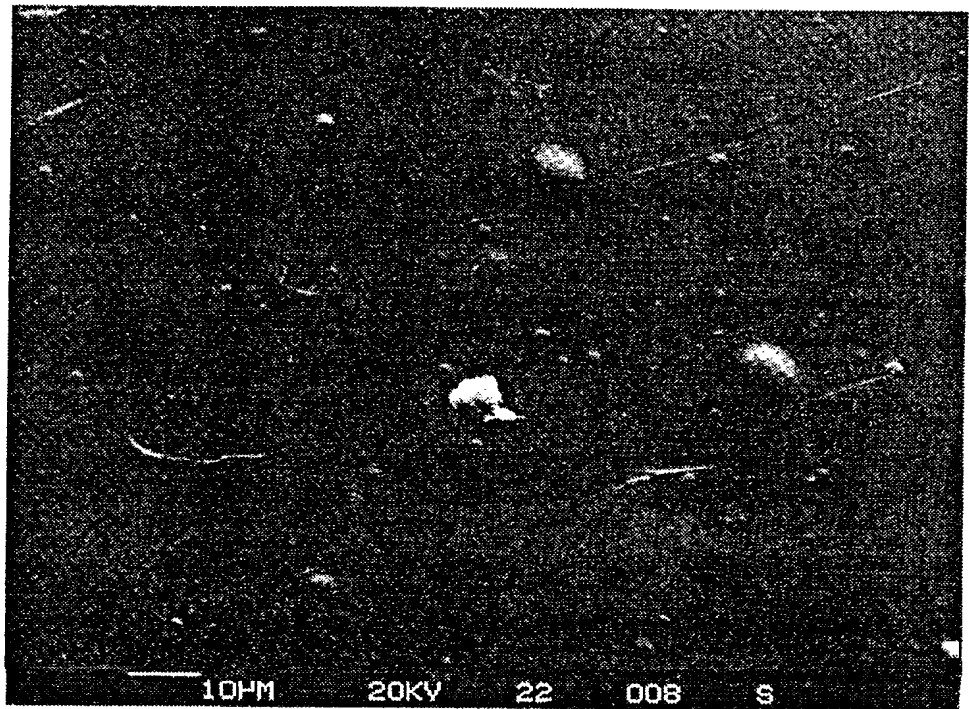
1/1

FIG. 1

a)



b)



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/01215

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C08F246/00; C09D157/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C08F	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US,A,3 671 502 (THE KENDALL CO.) 20 June 1972 ---	
A	DE,A,2 640 967 (NIPPON PAINT CO.) 5 January 1978 ---	
A	EP,A,0 293 963 (DOW CHEMICAL) 7 December 1988 -----	
<p>¹⁰ Special categories of cited documents : ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
19 OCTOBER 1992		29. 10. 92
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		CAUWENBERG C.L.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9201215
SA 61880**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 19/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-3671502	20-06-72	CA-A- 940648	22-01-74
DE-A-2640967	05-01-78	JP-C- 995920	30-04-80
		JP-A- 53000282	05-01-78
		JP-B- 54031520	08-10-79
		CA-A- 1113644	01-12-81
		GB-A- 1529378	18-10-78
		US-A- 4215028	29-07-80
EP-A-0293963	07-12-88	AU-B- 611143	06-06-91
		AU-A- 1472388	17-11-88
		JP-A- 1004230	09-01-89
		US-A- 4929666	29-05-90
		US-A- 5006624	09-04-91